A Dissertation on

COMPARATIVE STUDY OF AMNIOTIC MEMBRANE GRAFT AS AN ALTERNATIVE TO CONJUNCTIVAL AUTOGRAFT IN PTERYGIUM SURGERY

Submitted to the

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

In partial fulfilment of the requirements

For the award of degree of

M.S. (Branch-III)

OPHTHALMOLOGY

GOVERNMENT STANLEY MEDICAL COLLEGE & HOSPITAL

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY,

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APRIL 2013

CERTIFICATE

This is certify that study entitled **''COMPARATIVE STUDY OF AMNIOTIC MEMBRANE GRAFT AS AN ALTERNATIVE TO CONJUNCTIVAL AUTOGRAFT IN PTERYGIUM SURGERY''** is the result of original work carried out by **Dr.KIRTHI KOKA**, under my supervision and guidance at STANLEY MEDICAL COLLEGE, CHENNAI. The thesis is submitted by the candidate in partial fulfilment of the requirements for the award of **M.S Degree in ophthalmology**, course from May 2010 to April 2013 at the Stanley Medical College, Chennai.

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DECLARATION

I hereby declare that this dissertation **entitled "COMPARATIVE STUDY OF AMNIOTIC MEMBRANE GRAFT AS AN ALTERNATIVE TO CONJUNCTIVAL AUTOGRAFT IN PTERYGIUM SURGERY"** is a bonafide and genuine research work carried out by me under the guidance of **Prof. Dr.K.Kanmani**, M.S., D.O., Associate professor, Department of Ophthalmology, Government Stanley Medical College and Hospital, Chennai – 600 001.

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Title of the Work : Comparative study of aminotic membrance graft with Conjunctival auto graft in pterygium surgery using fibrin Thrombin Glue

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The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 13.07.2012 at the Council Hall, Stanley Medical College, Chennai-1 at 2PM

The members of the Committee, the secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

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PART - I

INTRODUCTION

Pterygium was recognised 3000 yrs ago. It was described by Susrutha way back in 1000 B.C. in India. Pterygium literally means "wing" and is an encroachment of the conjunctiva on thecornea, more often on the nasal side and is found in areas of high ultraviolet radiation, dry, hot, windy, dusty, and smoky environments[1]. There are usually no problems in diagnosing it but treatment can be difficult[2]. Currently it is believed that limbal stem cells and pterygial fibroblasts exposed to ultraviolet rays are damaged[3]. The indications for surgery include reduced visual acuity due to encroachment upon the visual axis and irregular astigmatism and also due to chronic irritation and recurrent inflammation, and cosmesis[4].

The treatment of choice is surgery[5] and various techniques exist with the resulting defect being left exposed or covered by surrounding conjunctiva or a limbalautograft or other tissues. Adjunctive therapy with anti-metabolites has been tried[6]. The use of amniotic membrane has been used as a viable alternative to conjunctivalautografts.

The need for conducting the present study is to evaluate the clinical presentation and effective management of primary pterygium with conjunctivalautograft and amniotic membrane graft.

REVIEW OF LITERATURE & HISTORY

The word pterygium comes from the Greek root "pterygios" which means "little wing". Susrutha an Indian doctor who lived in 1000 B.C. gave an accurate description of a pterygium and its treatment with pulverised salt and stimulation with a palm branch and when it was inflamed and swollen he tore it out with forceps and removed any remaining tissue with a flesh stripping ointment. He also described its recurrence[10]. Hippocrates and many other ancient greats like Celso, Galeno, Egineta and Avicenna described various approaches to the treatment of a pterygium[12,13].

Ambrose Pare described it as an abnormality which always recurs[8,9]. In the 18th century copper sulphate was used in treatment while in the 19th century silver nitrate and lead acetate and atropine were used. Surgery first appeared as a treatment modality in the 19thcentury[7,11,14].

Scarpa (1802): Removal of head from the cornea with a forceps and concentric excision of the detached tissue as far as the limbus[18].

Arlt (1850): Excision of the head from the head from the cornea and a diamond shaped portion of the body with conjunctival cross-over plastic surgery[19.]

Desmarres (1855): Technique of deviating the head towards superior fornix and inducing it to atrophy[20].

Knapp (1869): Technique of transposition[21.]

Arlt (1872): First scleral repair following excision of the pterygium[15,22].

McReynolds(1902): Modified Desmarres technique using a conjunctival pouch[16,23].

Gifford(1909): Epidermal growth to cover the sclera[24].

Morax and Magitot(1911): Used first artificially preserved homologous corneal grafts[25].

Terson (1911): Radiation therapy with X-Rays[26].

Fuchs (1911): Autologous penetrating keratoplasty and **Terson** (1913) used this for pterygia[17].

Magitot (1916): Suggested lamellar autokeratoplasty[27].

Amorin (1936): Suggested use of a diathermy coagulator[28,29].

D'Ombrain (1948): Technique of bare sclera[32].

Paufique (1950): Developed a lamellar keratoplasty[31,33].

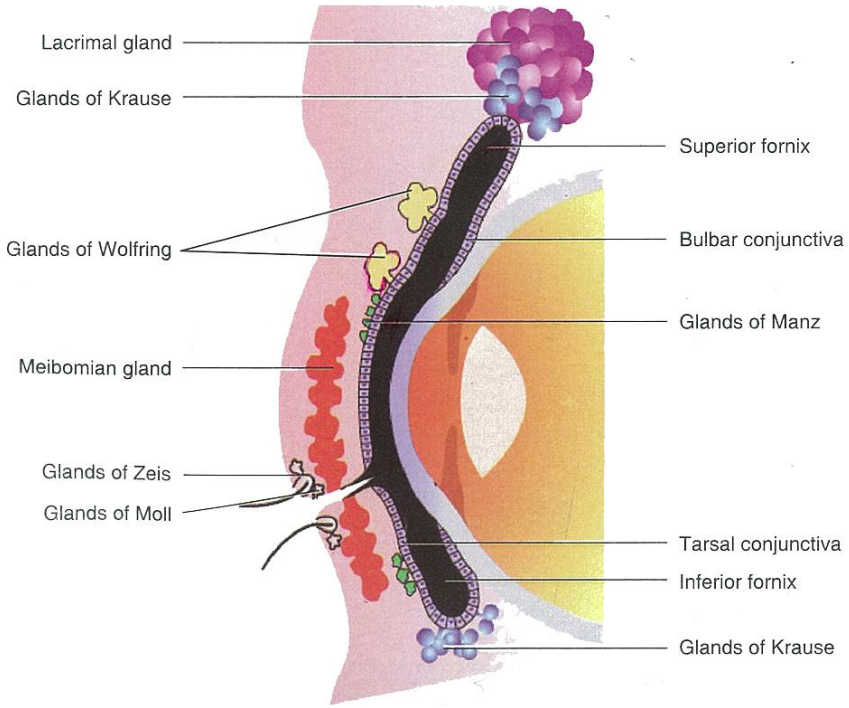
Haik (1957): Topical beta therapy with Strontium 90[30,34].

Meacham (1962): Used antimitotics to prevent recurrences[35].

Panzardi (1964): Used amniotic membrane for repair of defect[36].

Kenyon (1985): Grafting autologous conjunctiva to prevent recurrences[37].

Anatomy of conjunctiva



ANATOMY OF THE CONJUNCTIVA

The conjunctiva is a thin translucent mucous membrane which derives its name from the fact that it joins the eyeballs to the lids[38].

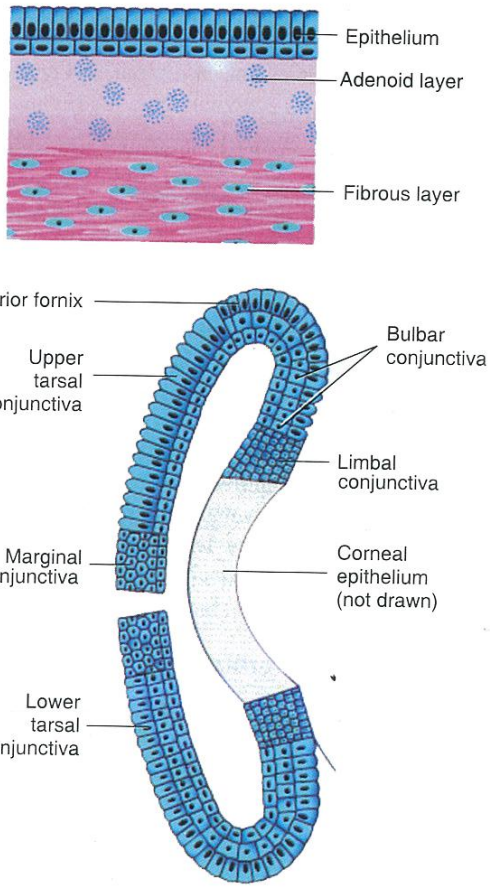
It lines the posterior surface of the lids and then is reflected forwards on to the globe of the eye. Its epithelium becomes continuous anteriorly with the epithelium of the cornea[39].

Thus it forms a complex space, the conjunctival sac which is open in front of the palpebral fissure, and closed only when the eyes are shut[40]. It has three regions:

1. Palpebral conjunctiva
2. Bulbar conjunctiva
3. Fornix conjunctiva

Since pterygium is confined to the bulbar conjunctiva this part is described in detail.

Structure of conjunctival epithelium



The Bulbar Conjunctiva:

It is a thin and translucent mucous membrane. It lies loosely on the underlying tissues, so that it can be easily moved apart from them.

It is loosely attached by connective tissue to the sclera and fascia bulbi. About 3mm from the cornea, the conjunctiva becomes more closely attached to the sclera and fascia bulbi. At the point of union, the conjunctiva is sometimes raised by a slight ridge, which becomes apparent in inflammatory conditions. This portion of conjunctiva is known as the limbal conjunctiva.

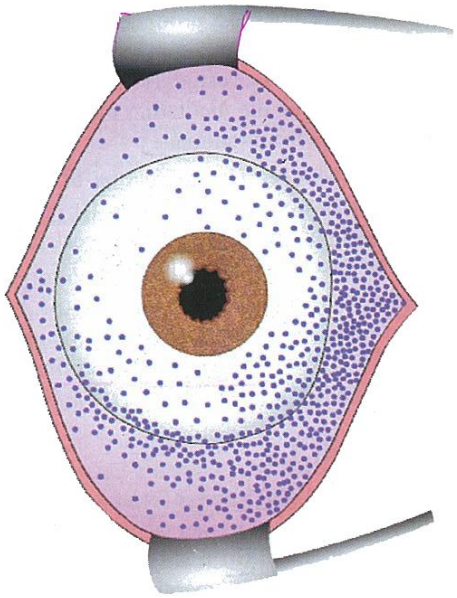
Structure Of Conjunctiva:

The conjunctiva like all other layers consists of two layers the epithelium and sub mucosal lamina propria[41].

Epithelium:

The epithelium consists of non-keratinised squamous cells arranged in five layers. The deeper layer consists of high cylindrical cells. This is followed by several layers of polyhedral cells,the most superficial cells are flattened. Near the limbus, the layers of squamous cells are gradually reduced and replaced by columnar and cubical cells. The total numbers of layers is also reduced[41].

Goblet cell distribution



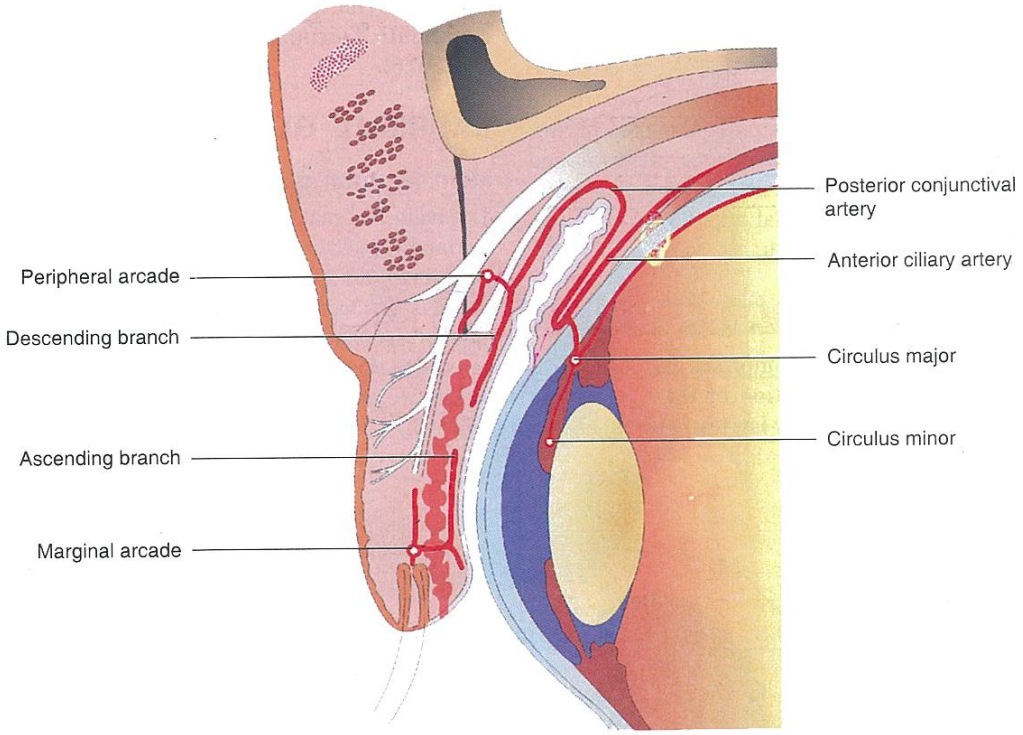
The epithelium of the tarsal conjunctiva of the upper eyelid consists of two layers. From the limbus to the fornix the epithelium becomes more and more glandular.

At the limbus the epithelium is definitely stratified with the formation of papillae that give a deep aspect of epithelium a characteristic wavy outline.Here the deepest or basal layer forms a single layer of cylindrical or cubical cells. The basal cells often contain pigment granules. Goblet cells occur in whole of conjunctiva including the plica semilunaris. They are large oval or round cells, which look like flat cells.

The goblet cells in the conjunctiva are greatly increased in number in inflammatory conditions. Associated with the conjunctiva are a number of small glands differentiated both histologically and topographically into different types. These are collectively called as conjunctival glands and distinguished only by eponymous names- the glands of Wolfring, the Henle's gland and the gland of Manz. These glands secrete mucin[41].

The conjunctival sub mucosa has a superficial lymphoid layer and a deeper fibrous layer. The lymphoid layer is not present at birth, but is formed first in the region of the fornix at the age of 3 to 4 months.

Blood supply of conjunctiva



It consists of fine connective tissue reticulum in the meshes of which the lymphocytes lie. The fibrous layer is generally thicker than the lymphoid. The vessels and nerves of conjunctiva are present in this layer.

Conjunctival Papillae:

True papillae are found only at the limbus and at the lid margin. Those near the limbus are finger like processes of sub mucosal tissue, the interspaces of which are filled with epithelium while the surface epithelium remains flat.

Blood Supply:

The arterial supply of the conjunctiva is from three sources:

1. The peripheral arterial arcade of the lid
2. The marginal arcade of the lid
3. The anterior ciliary arteries.

The peripheral arterial arcade is situated at the upper border of tarsus. It gives off peripheral perforating branches that pass above the tarsal plate and pierce the palpebral muscle to reach the conjunctiva, under which it sends branches upwards and downwards.

The marginal arcade sends its perforating branches through the tarsus to reach the deep surface of the conjunctiva at the subtarsal fold. These branches divide into marginal and tarsal twigs.

The anterior ciliary arteries are the branches of muscular arteries to the recti. Each muscular artery gives off two anterior ciliaries except that of lateral rectus which gives off one branch.

The anterior ciliary arteries give off the anterior conjunctival arteries, which pass forwards at a deeper level than posterior conjunctival vessels. They pass further and anastamose with each other and form a series of arcades parallel to the corneal margin and forms peri-corneal plexus. The peri-corneal plexus is arranged in two layers, superficial conjunctival and deep episcleral plexus[42].

Conjunctival Veins:

The conjunctival veins accompany, but are more numerous than the corresponding arteries. For the most part, tarsal conjunctiva, fornix and the major portion of the bulbar conjunctiva drain into palpebral veins.

Lymphatics:

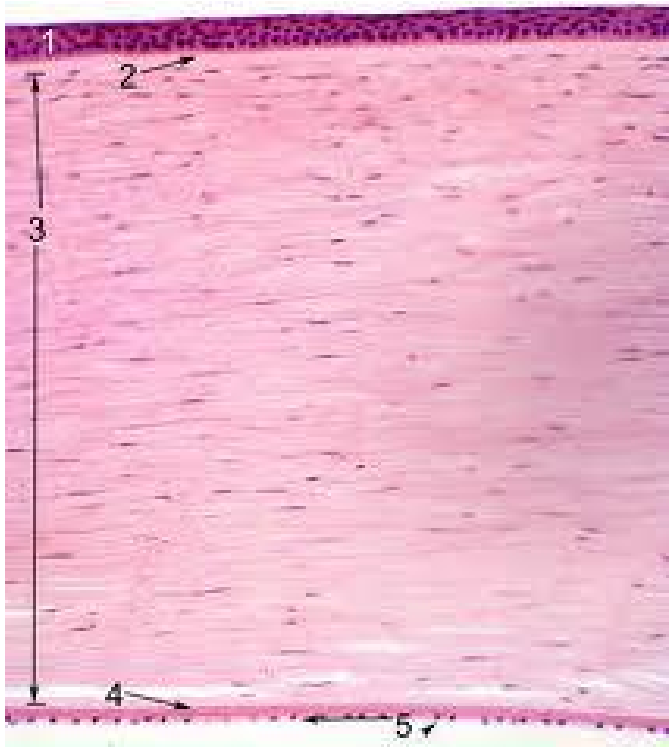
The conjunctival lymphatics are arranged in two plexuses. A superficial composed of small vessels, placed just beneath the vascular capillaries and consisting of larger vessels, situated in the fibrous layer of the conjunctiva and receiving the lymph from the superficial plexus.

They drain towards the commissures where they join the lymphatics of the lids ; those from the lateral side are drained by the pre auricular nodes and those from the medial side are drained by the sub mandibular nodes.

Nerve Supply:

The circum-corneal zone of conjunctiva is supplied by long ciliary nerve. The remaining part of the conjunctiva is supplied by the lacrimal and infra trochlear nerve[42].

Anatomy and histology of cornea



ANATOMY OF THE CORNEA

With the advent of the electron microscope, the following five layers have been identified.

1. Layers of stratified squamous epithelium
2. Bowman's membrane – the anterior limiting lamina
3. The substantia propria
4. Descemet's membrane – the posterior limiting lamina
5. The endothelium

Layer of Stratified Squamous Epithelium:

This layer is approximately 50 milli microns thick and consists of 5 to 6 layers of cells. The deepest of these, the basal cells are columnar. They are the germinal layer and are continuous at the corneal periphery with the same layer in the conjunctiva.

The next layer consists of polyhedral cells whose rounded heads are directed anteriorly and whose concave bases fit over the heads of the basal cells.

The next two or three layers are also polyhedral and most superficial are flattened but do not lose their nuclei, nor do they show keratinisation. The flattened nuclei of the surface cells project backwards leaving the surface perfectly smooth, which makes it the most brilliant in the body[43].

Bowman's membrane:

The anterior limiting lamina is a thin homogenous sheet about 8 to 14 milli microns thick between the basement membrane and the substantia propria.

The anterior limiting lamina is not truly elastic, nor does it regenerate when it has been destroyed. It however shows a good deal of resistance to injury or infection.

Electron Microscopy[44]: an acellular mass of collagen fibrils is disposed irregularly.

The SubstantiaPropria:

It is composed of modified connective tissue. It is about 0.5mm thick. The collagen fibrils are not visible under light microscopy.

Electron Microscopy[44]: the fibres are in alternating direction. They are parallel and in alternate layers they lie at right angles. In man, the collagen corpuscles line within and not between the collagen lamellae.

Descemet's Membrane:

The posterior limiting lamina, a thin elastic membrane covered on its posterior surface by endothelium. It is a strong and homogenous and a very resistant membrane. It is 10 to 12 milli microns thick. It is very resistant to chemical reagents and likewise to pathological processes going on in the cornea.

Electron Microscopy[44]: composed of very fine regular strata of fine collagenous fibres. In man they are disposed in two layers, an outer 'banded' against the substantiapropria and inner stroma against the endothelium.

The Endothelium:

Single layer of flattened hexagonal cells.The endothelium of the cornea can be seen by slit lamp in the living eyes, the only place in the body where this is possible.

In the corneal epithelium there are no glands. Embryologically the cornea is the continuation of three structures:

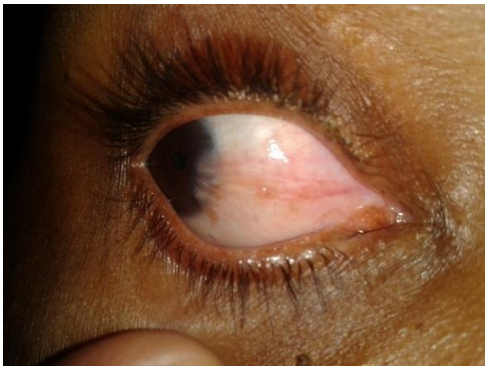
a. The epithelium and Bowman's membrane of the conjunctiva

b. The substantiapropria of the sclera

c. The endothelium of the uveal tract

Pathologically too, this is of importance, for the epithelium is liable to be affected in diseases of the conjunctiva, the stroma diseases of the sclera and the posterior lamina and endothelium in diseases of the uveal tract[45].

Pterygium – anatomy



ANATOMY OF THE PTERYGIUM

Macroscopic:

Pterygium consists of a head, an apex towards the advancing edge, neck and the body lying limbal to the head.

The Head:

The active part of the pterygium causing changes to the cornea in front of it, forming the cap and activates the subconjunctival connective tissue behind it forming the body. It ranges from fibrous, flat and avascular to the fleshy gelatinous type. The head is always firmly adherent to the underlying cornea.

The Cap:

This is the apex and slightly transparent with the cornea visible upto the Descemet's membrane. It is usually avascular[47].

The Neck:

The narrowest part of the pterygium, where the body and apex join.

The Body:

It is wing shaped and lies over the sclera. The adherent conjunctiva is dragged along with it and stretched so folds appear. There is always some degree of associated subconjunctival connective tissue hyperplasia[47].

The pigment line:

It is seen about a millimetre ahead of the cap. Iron from lactoferrin in the tears are deposited in the advancing edge of the pterygium as "*Stocker's line*.

Microscopic Structure[48]:

1. Fibroplastic tissue separating the corneal basement epithelial cells from the Bowman's membrane.
2. Altered orientation of the basal epithelial cells overlying the fibroplastic tissue.
3. Destruction of the Bowman's and superficial stroma beneath the fibroplastic tissue.
4. Normal corneal tissue proximal to the leading edge of the pterygium.

Characteristic features of Ocular Pterygia[49]:

a. Hyalinisation of the sub epithelial connective tissue of the substantiapropria

b. Increased number of thickened and tortuous fibres

c. Connections within the hyalinised and granular areas that may show either eosinophilia or basophilia.

DEFINITION AND CLASSIFICATION

The definition of pterygium varies according to the author.

Sir Stewart Duke Elder: true pterygium is a degenerative and hyperplastic process in which the conjunctiva actively invades the cornea. It is essentially a triangular encroachment of the bulbar conjunctiva into the cornea[7].

Robert G. Small: A pterygium is a triangular wedge of fibrous tissue covered by conjunctiva, which grows from the nasal limbus, slightly below the midline, onto the cornea, beneath the basement membrane of the corneal epithelium.

True pterygium: It is a degenerative and hyperplastic process in which the conjunctiva actively invades the cornea[48].

Pseudo pterygium: This is a condition in which a fold of inflammed conjunctiva becomes adherent to a progressive ulcer near the corneal margin and is passively drawn across the cornea.

True pterygium and pseudo pterygium

True pterygium **Pseudo pterygium**

DIFFERENCE BETWEEN TRUE AND PSEUDO PTERYGIUM

TRUE PTERYGIUM	**PSEUDO PTERYGIUM**
Probe cannot be passed	Probe can be passed
Nasal and temporal aspects	Any part
Degenerative and hyperplastic process	Inflammatory process
Usually single	May be multiple
Recurrences seen	No recurrence
HISTORY	**HISTORY**
No h/o injury, chemosis or inflammation	History present
AGE	**AGE**
After 20-25 yrs	Any age
SHAPE	**SHAPE**
Wing shaped	No typical shape
GROWTH	**GROWTH**
May progress, regress, be stationaryor atrophy	Remains stationary
Stoker's , Bussacca's lines seen	Not seen

Different authors have classified pterygium in different ways:

WILLIAM M. TOWNSEND CLASSIFICATION[50]:

A. Actively growing

B. Slow growing

C. Fleshy or malignant

D. Stationary

E. Atrophic

LUCIO BARRATO'S CLINICAL CLASSIFICATION:3 main clinical types

Type 1: Small primary pterygium[51]

These are initial forms of pterygia with very mild or no symptoms.

a. Fibrous :

- Small whitish or yellowish circles parallel to the limbus
- Conjunctival blood vessels converging towards the circles
- The body is not clearly distinguished

b. Pinguecular:

- Appearance similar to a pinguecula, protruding
- Head can just about be identified and does not invade cornea
- 2-3 mm of stromal infiltration

c. Classical:

- All portions can be clearly identified
- Apex invades 1-2mm of the cornea

Type 2: Advanced Primary or Recurrent pterygium with no optical Zone involvement[51]

Irritation and initial reduction in vision occurs due to irregular astigmatism. The cornea is invaded for 2-4mm and reaches the optical zone. The body is thickened with dilated, congested capillaries. The corneal infiltration can be seen with the naked eye.

Type 3: Advanced Primary or Recurrent pterygium with Optical Zone involvement[51]*:*

There are obvious symptoms with serious reduction in vision. There may be oculo-motory disturbances and a watery eye syndrome. The head invades the cornea for more than 4mm and reaches the optical

zone. There is always a reduction in vision, and the collarette is extended (up to 8-10mm) and the body extends up to the entire medial canthus. There is abundant sub-conjunctival fibrosis which extends as far as the fornices.

GRADING OF PTERYGIUM BASED ON EXTENT[52,53]:

Based on the amount of corneal encroachment, the pterygium is graded as follows[45]:

- Grade I – crossing the limbus
- Grade II – midway between the limbus and pupil
- Grade III – crossing the pupillary margin

INCIDENCE

Pterygium is a common ophthalmic condition of tropics and sub tropics. It is a condition found commonly in sunny, hot, windy coastal regions of the world, mostly between the latitude of 32° north and south of the equator. The incidence also varies with the amount of exposure to climactic conditions. It is most common in those who work outdoors and therefore more among men than women (2:1) except in localities where the exposure for both are equal. In some communities its incidence is almost exclusively confined to rural workers and fishermen[42].

Site:

It affects mostly the nasal part of the conjunctiva and occasionally is present on both sides. It may affect one eye or both eyes. The normal flow of tears is from the temporal to nasal side towards puncti. It carries dust particles to the conjunctival sac and accumulates in the lacus lacrimalis. These concentrated dust particles may cause greater irritation of nasal conjunctiva.

Age and Sex Distribution:

It is more among males and increases with age. It affects adults between 20 and 40 years of age. It is rare among children.

Occupation:

It is most common among people who are exposed to effects of long standing irritation. Thus it is common among stonecutters and farmers. Hence people who are exposed to outdoor work, dust, wind, smoke, heat and bright light are prone to get pterygium[26].

Environmental Factors:

This condition is more common in localities where the weather is to the hot side and the sunlight is brilliant. This explains the frequency of pterygium among stonecutters and inhabitants of certain localities.

AETIOLOGY AND HISTOPATHOGENESIS[12,32,45]

The biological and pathogenic factors that cause pterygia are not well understood and it is unknown why recurrences occur in some inspite of receiving the same treatment strategies.

HEREDITARY FACTORS

A pterygium may be hereditary and run in families with a dominant gene of incomplete penetrance. This refers to a predisposition of the conjunctiva to an abnormal response to atmospheric or environmental stimuli.

PINGUECULAR

Suggested by Fuchs and expanded by Guillermo Pico and others[47]. According to this theory the primary lesion is a pinguecula. The microerosions caused at the limbus by environmental or lacrimal factors, provokes a defence reaction in the conjunctiva. When this lesion involves the cornea it produces edema which causes the migration of limbal keratoblasts, the so called "*advancement front*" of the lesion or "*Fuch's progressive area*".

INFLAMMATORY

Arlt, Scarpa, Hirschberg, Von Grafae and Kamel have supported this theory which states that erosions and micro erosions provoked by environmental and professional stimuli at the limbus incites sub clinical inflammation in the conjunctiva[25].

ANOMALIES OF TEAR FILM

Discontinuity of the tear film with formation of small dellen and epithelial micro ulcerations is the initial stimulus and this was emphasised by Barraquer[61].

DIET / NOURISHMENT

By Beard and Dimitry stating that a deficiency of Choline and Vitamin A is the cause.

ANGIOGENIC TISSUE FACTORS

Wong stated that repeated irritation of the limbus produces an angiogenic factor which gives rise to a pterygium.

IMMUNE MECHANISM

Suggested by Hilgers[56] in the sixties. Due to imbalance in cell mediated immunity, a prevalence of CD3 lymphocytes with suppressor activity is present. The ratio between helper and suppressor cells in the normal conjunctiva is 1:1.5 while in pterygia the mean ratio is 1:2.7. In addition mast cells, plasma cells and deposits of immunoglobulin with the characteristic granular pattern may be seen.

ENVIRONMENTAL ULTRAVIOLET RADIATION

This is currently the most credible theory. Kerknezov (1956) found that an outdoor life was essential for pterygium formation. Cameron mapped the incidence rates of pterygia and showed the disease was concentrated in preequatorial regions and increased towards the equator.

The working hypothesis is that UV rays cause mutations in the p53 tumour suppressor gene and this facilitates abnormal proliferation of limbal epithelium[54].

PATHOLOGY

The pathogenesis of pterygia is not fully understood and recent evidence implicating genetic components, anti-apoptotic components, cytokines, growth factors, extracellular matrix remodelling, immunological mechanisms and viral infections in the pathogenesis of the disease.

Low levels of pigment epithelium derived factor and increased vascular endothelial growth factor levels have been implicated in the pathogenesis[45].

Pterygium epithelial cells also show a unique feature of positive immune histochemical staining pattern for different types of matrix metalloproteinases[53]. Evidence shows that pterygia originate from invasion of Vimentine expressing altered limbal epithelial basal cells[4].

DIFFERENTIAL DIAGNOSIS

1. PSEUDOPTERYGIUM – situated anywhere and is due to an inflammatory process.

2. PINGUECULA – seldom encroaches on the cornea except when it is very large.

3. EPITHELIOMA –it has a more irregular surface, lacks thickened sub conjunctival connective tissue at the caruncle and there is an absence of orientation of blood vessel into the characteristic pterygial shape.

4. BOWEN'S TUMOUR – rare tumour with features similar to epithelioma.

5. EPITHELIAL HYPERPLASIA – increase in subconjunctival connective tissue with white or grey plaques surrounded by erythema mimic a pterygium.

6. SQUAMOUS CELL CARCINOMA OF THE LIMBUS – it is very rare but hard to differentiate from a pterygium at times. It is more common in the infero-temporal zone of the limbus. The definitive diagnosis is by histological examination.

7. CONJUNCTIVAL PAPILLOMA – it is highly vascular and bleeds easily and is of a viral origin.

8. LIMBAL DERMOID – rare congenital pathology seen mostly in the infero-temporal sector.

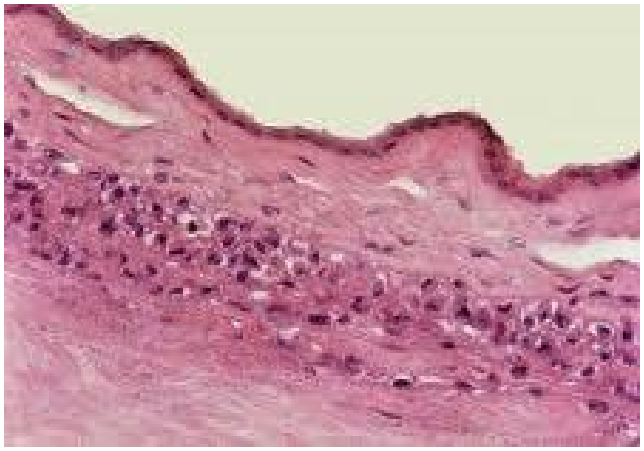
9. PHLYCTENULAR KERATOCONJUNCTIVITIS – small circumscribed conjunctival neo- formation with a gel like appearance and surrounded by twisted capillaries and associated with conjunctival hyperemia.

10. LYMPHOMA OF THE CONJUNCTIVA – very rare and is a salmon pink sub-conjunctival lesion mainly on the inferior and nasal bulbar conjunctiva.

11. NODULAR EPISCLERITIS – localised nodular forms appear as a bright red almost flat nodule. It is seen more often in young adult females.

The differential diagnosis of pterygium should also include conjunctival intraepithelial neoplasia, squamous cell carcinoma, a corneal micro pannus etc. The characteristic features of these clinical entities will help to distinguish them from a pterygium.

Structure of amniotic membrane



AMNIOTIC MEMBRANE

Amniotic membrane is the innermost semi-transparent layer of the foetal membranes. It has an avascular stromal matrix, a thick collagen layer and an overlying basement membrane with a single layer of cuboidal epithelium. Amniotic membrane, used as a graft initially in 1940[54], gained popularity in reconstructive surgeries since 1995.

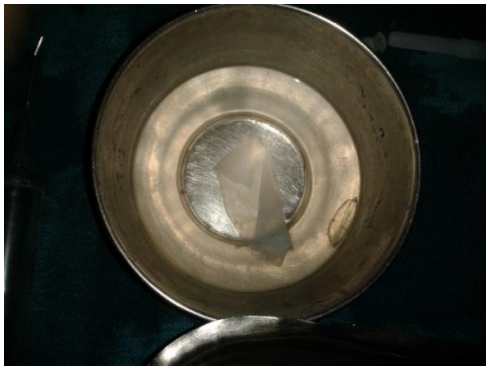
Properties of Amniotic Membrane[54]:

The biological properties of human amniotic membrane (AM) in relation to its transplantation on to ocular surface

Biological properties of AM	Relationship to transplantations
Immunogenic character	
Expression of incomplete HLA-A,B, and DR antigens	Rejection of AM does not occur
Human amniotic epithelium does not express HLA antigens	Rejection of AM does not occur
AM grafts transplanted to Lewis rats were accepted, but not the skin grafts.	AM is immunologically privileged
Promotion of healing	
Down regulation of TGF β signaling	Promotion of anti-fibrotic effect & anti-scarring effect.
Stromal matrix of AM suppresses lipopolysaccharide induced upregulation of IL-1 α & IL-1β	Reduction of surface ocular inflammation resulting in reduced scarring & metaplastic changes.
Preserved human AM express mRNAs for epidermal growth factor, keratocyte growth factor, hepatocyte growth factor fibroblast growth factor, transforming growth factors (TGF) α & β	These factors play important role in epithelialization & healing.
Preserved AM suppress expression of TGF βI, II, III, TGFb receptor type II.	Suppression of myoblast differentiation in corneal & limbal fibroblasts.
	Inhibition of extracellular matrix production and scar formation by these fibroblasts.
Preserved AM suppresses signaling pathway of TGFβ, CD-44, β-1 integrin & FGFR1/flg of pterygium	Felicitates migration & adhesion of corneal epithelial cells and proliferation of keratocytes. AM acts as a "contact bandage lens" allowing epithelialization to occur under its cover.
Proteins in the matrix are present in the preserved AM	Prolongation of the life span & clonogenicity of epithelial progenitor cells, promotion of goblet cell differentiation.
Other mechanisms	
Expression of chemokines by fibroblasts & interleukin 1 by epithelial cells are inhibited by preserved AM.	Inhibition of inflammation.
Preserved AM possesses anti-angiogenic factors, anti-inflammatory proteins & protease inhibitors	Inhibition of vascular endothelial growth & prevention of neovascularization
AM acts as a mechanical barrier	Functions as an anatomical barrier keeping the potentially adhesive surfaces apart

Courtesy: IJO 2005

Amniotic membrane procurement



Preparation[55,56]:

The following steps may be followed to finally obtain quality AM for transplantation. The required transport medium, the washing solutions and preservative medium, properly checked for sterility should be made available at least a day before the collection and preparation of the membrane. The working solutions and media should have been prepared 7 - 10 days ahead with complete verification of pH and sterility. It is preferable to prepare these solutions when required. The preparation is described below:

- The detailed medical history and clinical condition of the potential donor should exclude the risk of tissue-transmissible infections and unsuitability of the donors.

- Donors are screened for human immunodeficiency virus (HIV) type 1 and 2, hepatitis B virus (HBV), hepatitis C virus (HCV) and *Treponema pallidum* infections.

- The Amniotic Membrane should be obtained under sterile conditions after elective caesarian section.

- The Amniotic Membrane is dissected from the placenta in two large bits. As much of the chorion as possible should be peeled out

before the bits are dropped into a sterile, wide mouthed 125 ml screw-capped reagent bottle containing 50 ml transport medium. The transport medium generally used is the commercially available Eagles' minimum essential medium (EMEM) supplemented with 3.3% L-glutamine and antibiotics (50 μg/ml gentamicin, 100 units/ml penicillin, 200 μg/ml ciprofloxacin and 1 mg/ml Amphotericin B). The membrane must be transported immediately to the laboratory.

- In the laboratory, under the laminar flow hood, the Amniotic Membrane is washed free of blood clots with EMEM containing antibiotics.

- With the epithelial / basement layer surface up, the Amniotic Membrane is spread uniformly without folds or tears on individually sterilised 0.22 μm nitrocellulose membranes of the required size.

- The Amniotic Membrane with the filter membrane is placed carefully in the preservative medium in 50-ml wide mouthed screw-capped irradiated transparent plastic bottles. The preservative medium used is 1:1 (vol/vol) ratio of sterile glycerol (sterilized by autoclave) and EMEM with 3.3% L-glutamine, 25

μg/ml gentamicin, 50 units / ml penicillin, 100 μg/ml ciprofloxacin and 0.5 mg/ ml Amphotericin B.

- Thedevitalization of the epithelial cells of the Amniotic Membranes is done by storing at -80°C.

- The membrane is thawed by keeping the bottle either at 4°C for 30 minutes or at room temperature for 10 minutes.

- Microbial contamination should be avoided and the storage medium should be light pink on thawing as opposed to yellow which indicates contamination.

- Before usage, the epithelial side can be identified by its stickiness to a cotton swab.

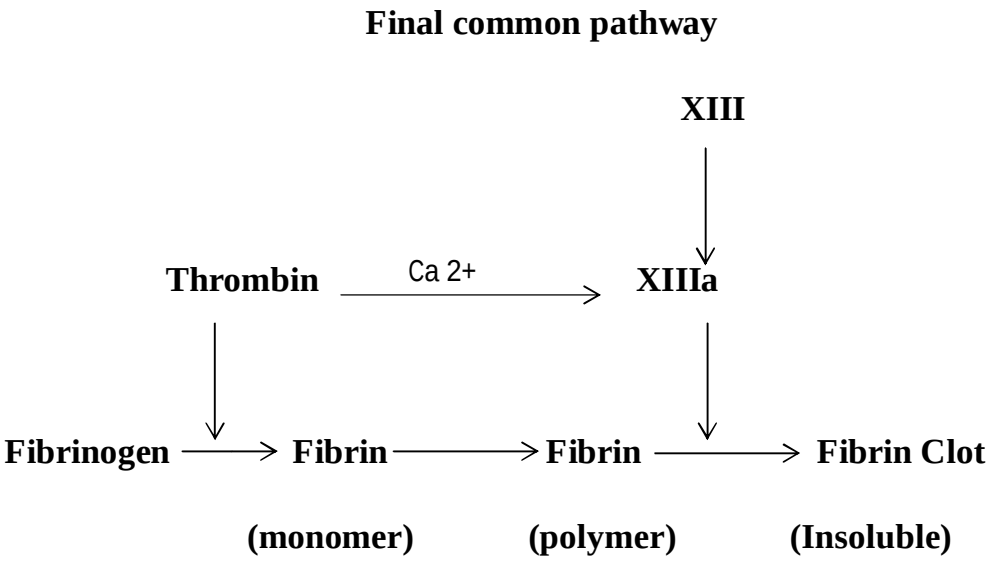
FIBRIN GLUE

Suturing is a time consuming process and may cause redness and irritation. Hence nowadays it is being increasingly replaced by tissue adhesives like Fibrin Glue, a blood derived product which is absorbable and easy to use. It acts as a smooth seal along the wound edges and it can be stored at room temperature. It is obtained as a commercially available preparation and may be prepared in blood banks[58].

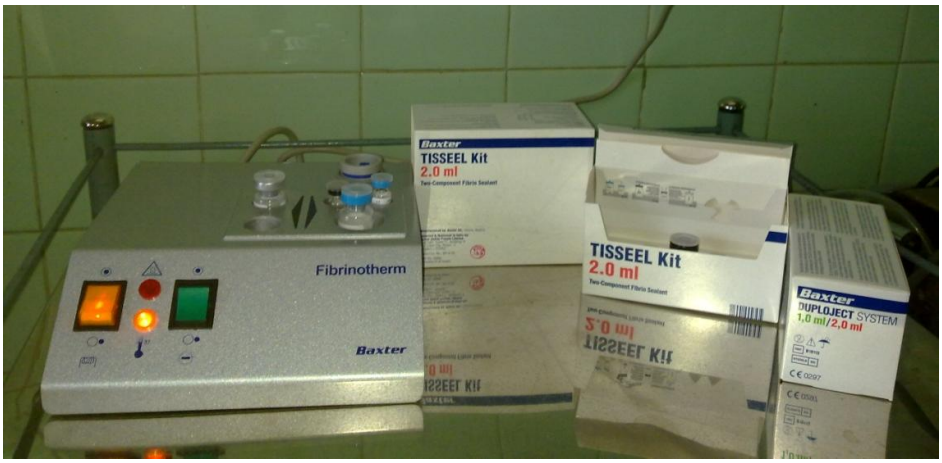
Components

1. Fibrinogen component
2. Thrombin component

Mechanism of action



Fibrin glue


Fibrinotherm
Baxter
TISSEEL Kit
2.0 ml
Two-Component Fibrin Sealant
DUPLOJECT SYSTEM
1,0 ml/2,0 ml


Baxter
TISSEEL 2.0
Thrombin-4


Fibrinotherm
Baxter

Method of preparation

A precipitate having fibrinogen and a thrombin containing supernatant is obtained after centrifugation.

Commercially available fibrin is:

Tisseell VH Fibrin Sealant (Baxter, AG, Vienna, Austria) – FDA Approved

Preparation:

For its preparation the cold chain has to be maintained. Color coded vials are first warmed in a patented fibrotherm device. Then the fibrinolysis inhibitor (aprotinin) is added to the sealer protein concentrate and a second component is prepared by injecting calcium chloride vial into thrombin vial and warmed. Only a small amount of thrombin – calcium chloride complex is needed to drive the reaction.

Then 0.1 ml of thrombin calcium chloride solution is diluted with 0.9 ml of balanced salt solution to achieve 1:10 dilution. A syringe is then placed into the duploject injector along with a parallel syringe containing fibrin sealer protein and fibrinolysis inhibitor. A mixer

nosecone topped by blunt applicator needle is attached to the 2-syringe nozzle. Then the common plunger is depressed causing fibrin sealer solution and thrombin solution to mix in equal volume.

A dry surgical field is required for application and the tissue must be left undisturbed for three minutes for firm adhesion.

Techniques of Application:

1. Simultaneous Application
2. Sequential Application

Advantages:

1. It reduces the total surgical time
2. Lower risk of post-operative infection
3. Mixtures of fibrin glue and antibiotic – helps in local delivery of antimicrobial activity
4. Smooth seal along entire length of wound with higher tensile strength
5. Useful adjunct to control bleeding
6. Low incidents of allergic reactions

MANAGEMENT

Is usually conservative, unless the following situations arise[7,21,25,27]:

- Decrease in vision due to mixed astigmatism or growth extending on to the visual axis.
- Cosmesis.
- Foreign body sensation and irritation not responding to medical treatment.
- Limitation of extra ocular movements due to restriction.
- Progression of growth towards the visual axis suggesting that visual loss will ultimately occur.

Pterygium induced astigmatism can lead to visual disturbances. It is usually with-the-rule astigmatism. It produces tear film abnormalities and it also exerts a mechanical pull on the cornea. But it is incorrect to label the entire astigmatism as “induced” as many may have a natural component too[58].

With increase in size, the induced astigmatism also proportionately increases. A pterygium larger than 3mm to 3.5mm (more than halfway to the centre of the pupil) are likely to be associated

with > 1 D of astigmatism [59]. The improvement in vision after surgery may be due to two causes:

a. Reduction in astigmatism, and

b. Removal of pterygium from the visual axis.

MEDICAL APPROACH

General recommendations for the prevention of pterygia should include the avoidance of exposure to ultra violet radiation.

Studies have shown that the development of pterygium is strongly associated with exposure to UV rays in the first five years of life and hence parents should be advised to protect their children from UV ray exposure, especially if the residence is within 30° of the equator.

The use of ultraviolet absorbing protective spectacles, sunglasses and hats is advisable.

Mild irritative symptoms due to pterygium may be managed with topical lubricants or a mild topical vasoconstrictor. A mild topical corticosteroid (eg.Fluorometholone 0.1% QID) may be useful for moderate to severe vascular injection and irritative symptomatology.

Secondary dellen may be managed with preservative free lubricating ointments and temporary patching for 24 hours [21,25].

SURGICAL APPROACHES

Ideally a pterygium surgery should have low or no recurrence, with minimal complications and be cosmetically acceptable. No single approach has been found to be perfect and each has its own advantages and disadvantages. All procedures regardless of adjunctive measures employed begin with the surgical removal of the pterygium from the globe. Dissection is either done from the body to the head or vice versa. The various approaches are:

EVULSIONS

A horse hair or flax thread or even squint hooks and corrugated silver wires have been used for dissecting the pterygial head from the underlying corneal tissue.

TRANSPLANTATION OF THE HEAD

Various techniques redirect the head of the pterygium away from the cornea to prevent recurrences. They consisted of burying the head

underneath the normal conjunctival edge inferiorly. But with recurrence rates of 30-75 % they have been largely abandoned[20,35].

PTERYGIUM EXCISION / AVULSION

Avulsing a thin, relatively transparent, primary pterygium by mechanically shearing off the head from the underlying cornea has been tried. It has the advantage of resulting in a smooth corneal surface, rapid epithelialisation and minimal scarring. But many pterygia cannot be avulsed and have to be excised[59]. Deep lamellar keratectomies offer no distinct advantages.

STEPS OF PTERYGIUM EXCISION

A reliable method described by Kenyon et al. consisted of the following steps.Peribulbar anaesthesia and a lid block are usually used but for a simple excision, topical Lignocaine and a sub-conjunctival injection of Lignocaine may suffice.

A rigid lid speculum is used. Limbal stay sutures are placed at 12 o'clock and 6 o'clock positions. The head is dissected away from the cornea by tenting the pterygium apex with the fine forceps and then performing a delineating keratotomy at the leading edge with a rounded sharp blade to obtain a superficial plane of dissection. The remainder of

the pterygium head is carefully dissected from the superficial cornea in a lamellar fashion up to the limbus with a Tooke's knife. Excision of the bulbar conjunctival extent of the pterygium is carried out up to the limbus using blunt dissection with Westcott's scissors. The pterygium is then excised from the remaining limbal attachment with scissors. All involved conjunctiva, underlying Tenon's capsule and scar tissue are ultimately removed down to bare sclera[60].

Care must be taken to avoid damage to the underlying rectus muscle, which can be enmeshed in the fibrovascular tissue (especially in recurrent cases). Wet field cautery is used to cauterize the bleeding vessels as necessary.The exposed bulbar conjunctiva margins are then tacked down to the sclera with several 10.0 nylon sutures (some advocate 8.0 vicryl sutures) with attention not to recess or advance the margins excessively. At this point the surgeon can proceed with conjunctival auto grafting for either primary or recurrent pterygium[40].

BARE SCLERA TECHNIQUE

Here the resultant scleral and corneal defects would be left to epithelialize post-operatively (d'Ombrian 1948). It was theorised that a pterygium recurrence would be prevented if the corneal epithelium could heal before the conjunctival epithelium reached the

limbus[49,50].Unfortunately Youngson reported a recurrence rate of 37%[52]. And Ehlers reported upto 91% recurrence in combination with excimer laser corneal ablation to smooth the corneal surface[61].

CONJUNCTIVAL FLAPS AND CONJUNCTIVAL AUTOGRAFTS

Healthy conjunctival tissue when approximated to the limbus following excision of a pterygium is associated with a low recurrence rate. Three basic variations on this theme exist:

Pterygium excision with Primary Conjunctival Closure

Primary conjunctival closure is achieved by undermining adjacent normal superior and inferior bulbar conjunctiva and pulling the cut conjunctival edges together to achieve primary conjunctival closure. Patient aged less than 40 yrs and those with aggressive pterygium activity are risk factors for recurrences[19,27].

Pterygium Excision With Rotational Flap

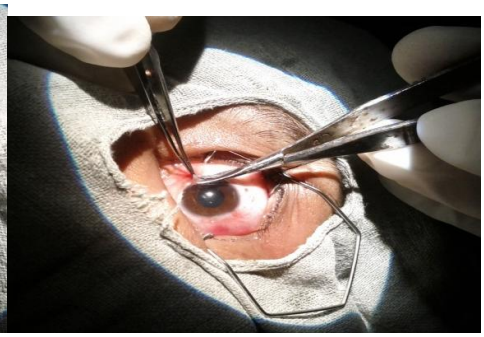
Rotational conjunctival flaps to cover the pterygium excision site have been employed since the 1940s. *Aratoon* in 1967 reported a recurrence rate of less than 1% in a series of 150 consecutive procedures by using a conjunctival pedicle flap after pterygium resection[62]. A report by *Wilson and Bourne* discussed a re-directional conjunctival flap

technique originally known as a *Z-plasty*[63], the procedure involved rotating a flap of normal conjunctiva into a limbal position while simultaneously rotating the remaining pterygium body laterally onto the bulbar conjunctiva after resecting the head from the cornea. Two advantages of this procedure include the preservation of normal conjunctiva for possible future free grafting and normal conjunctival tissue next to the limbus acts as a barrier to prevent recurrence.

Pterygium Excision With Conjunctival Autograft

Kenyon et al described conjunctival free graft transplant as a treatment for pterygium in 1985. This technique utilises a free conjunctival graft from the superotemporal bulbar conjunctiva to cover the exposed scleral surface after pterygium excision. A 5.3% recurrence rate was reported. The authors recommend this treatment modality for advanced primary and recurrent pterygium, especially when concurrent fornix reconstruction is required or when conjunctival scarring involves the extra ocular muscles. The importance of including limbal tissue in the conjunctivalautograft, to reduce the recurrence rate, has been described by Rao SK et al. This method decreases the chances of scleral necrosis seen with other adjunctive therapies [40].

Surgical steps for conjunctival autografting

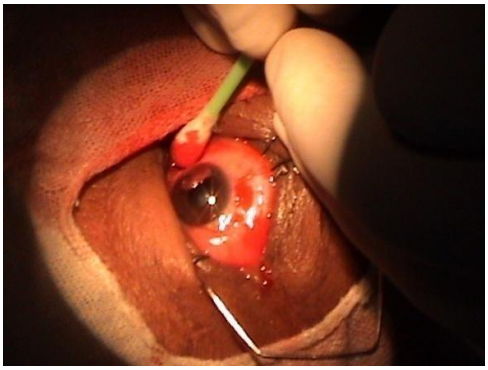


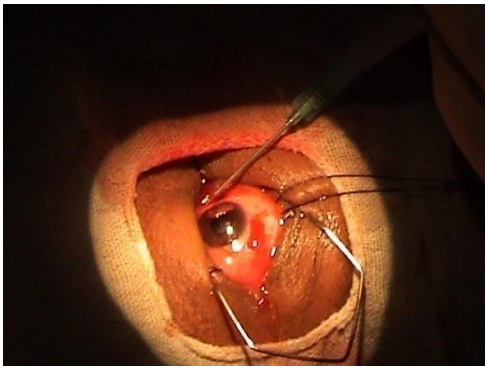
Procedure for conjunctival autograft surgery:

After the excision of the pterygium, the size of the scleral defect created is measured with Castroviejo callipers. The dimensions of the intended conjunctival graft are marked with gentian violet marking pen.The gentian violet marks aid not only in the excision of an approximately sized donor graft but also are invaluable in preventing inadvertent upside down orientation of the graft in the recipient bed.

Balanced salt solution is then injected sub-conjunctivally outside of the gentian violet marks to elevate the conjunctiva to aid in the conjunctival dissection. Blunt Westcott's scissors are used to incise the posterior border of the graft. The conjunctiva is then undermined using blunt dissection with care taken to not include the underlying Tenon's capsule in the final graft. The lateral edges of the donor graft are incised outside of the gentian violet marks as the dissection is carried forward. The donor conjunctival graft should be as thin as possible so that post-operative healing will occur with less shrinkage. It is also important that the limbal conjunctiva is incised last after the entire graft has been dissected forwards to the limbus. This assures that the graft will not retract and become difficult to handle. Handling of the donor

Graft fixation with fibrin glue





conjunctival tissue only occurs with non-toothed forceps (eg.McGregor conjunctival forceps),so as to avoid a buttonhole in the conjunctiva.

At this point, the graft is oriented with the unmarked limbal donor edge adjacent to the limbus in the recipient bed and the gentian violet marks on the exposed surface of the conjunctiva. The graft is secured with 10.0 nylon(buried knots) to avoid a post- operative graft dehiscence. Graft fixation can also be achieved with fibrin glue[62].

The majority of these sutures usually extrude by their own within one month of the surgery, while the rest epithelialize and remain buried. Patient discomfort is usually not a problem due to the usage of permanent sutures. The occasional exposed stitch can be removed after adequate conjunctival healing in the early post-operative period.

The donor harvest site is left to epithelialize on its own, which usually occurs in the first several post-operative days. Post-operative steroid and antibiotic drops six times a day during the first 1 or 2 weeks and switch to a steroid drop alone after that time. Lubricating drops may be used 4-6 times per day for 4-6 weeks.

The main drawback of the conjunctival free graft technique is the increased surgical time required, compared to other bare sclera or

primary closure techniques. Additionally an operating microscope is required for optimum results. Disadvantages are outweighed by the lack of sight-threatening complications and the relatively low recurrence rates after conjunctival free grafts[63].

Complications Of Pterygium surgery:

These are infrequent and not generally sight threatening[7,16,21].

Intra-operative

- Haemorrhage
- Injury to the medial rectus muscle
- Loss of orientation
- Donor site haemorrhage
- Button holing of the graft
- Dipping of the graft

Post-operative

- Conjunctival graft edema
- Corneoscleral dellen
- Epithelial inclusion cysts

- Tenon's granuloma – usually in the first 2 weeks. It is often associated with recurrence and should be excised.
- Subconjunctival fibrosis at harvest site causing EOM restriction and diplopia
- Graft retraction
- Giant papillary conjunctivitis due to suture irritation
- Symblepharon formation more with inferior donor sites

Lamellar keratoplasty and penetrating keratoplasty

If significant corneal thinning is present as a consequence of previous pterygium surgery, lamellar keratoplasty may be indicated to restore the normal ocular surface integrity. Lamellar keratoplasty acts as a barrier to pterygium regrowth. In severe cases where the visual axis is affected by thinning and scarring, penetrating keratoplasty may be indicated to visually rehabilitate the eye[64].

Mucous membrane grafts and skin grafts

Other grafting substances include buccal mucous membrane, skin taken from the flexorsurface of the forearm, behind the ear or from redundant skin of the upper lid. In cases in which sufficient conjunctiva

Amniotic membrane – intra operative

is not available for a pedicle graft, *Trivedi et al* recommended the use of a mucous membrane graft from the lower lip after pterygium excision[62].

The clinical circumstance of generalised conjunctival disease preventing rotational flaps or free grafts is uncommon. A split thickness skin graft decreases the incidence of recurrences in cases of secondary recurrent pterygia and presents an acceptable "white" eye post-operatively. The cosmetic appearance of skin grafting does not approach the excellent results of conjunctival rotational flaps or autografts[48]. Based on the paucity of reports, using skin grafts, the technique has not received widespread acceptance.

AMNIOTIC MEMBRANE GRAFT PROCEDURE

After the excision of the pterygium, the defect is then closed with human processed amniotic membrane. Amniotic membrane of required size is taken and then placed over the bare sclera with the basement membrane side up. The amniotic membrane is sutured to the episcleral tissue at the edge of the bare sclera border with 10-0 nylon sutures. Amniotic membrane fixation could also be achieved with fibrin glue. The patients were prescribed Prednisolone acetate eye drops that were tapered off 1 month following surgery along with lubricants for 4 wks. Antibiotics were given in the immediate postoperative period for 2wks.

ADJUNCTIVE THERAPY

In an effort to lower the recurrence rates after pterygium excision, several adjunctive treatment modalities have been developed.

The following are recommended for both advanced primary and recurrent pterygium.

THIOTEPA

It is a nitrogen mustard analogue in use since 1962. It is an alkylating agent that interferes with normal mitosis and cell division in all rapidly proliferating tissues[65].

Common recommended form is to mix 15mg Thiotepa in 30ml of Ringer's solution for a final dilution of 1:2000 strength. The medication is used topically every 3 hours during the day starting 2 days post-operatively for a total of 6-8 weeks time.

Complications include early and late onset poliosis and periorbital skin depigmentation that can become permanent, a major reason for it not gaining widespread acceptance.

MITOMYCIN C:

It acts by undergoing reduction activation and then it interacts with DNA to form monofunctional adducts as well as covalent crosslinks.

The use of topical Mitomycin C eye drops of 0.2-0.4 mg/ml strength 2-4 times daily was advocated to prevent pterygium recurrence.

Alternatively Mitomycin C can also be applied to the bare sclera left behind after pterygium excision. 0.25 mg/ml of MitomycinC is applied to the bare sclera directly for 1-3 minutes. Recurrence rates with 0.2-0.4 mg/ml Mitomycin was in the range of 5-9% [63,59].

Complications mainly include scleral ulceration, necrotizing scleritis, scleral calcifications, infections, severe secondary glaucoma etc.

RECURRENCE

The perfect result of a pterygium excision would be a normal appearance of the conjunctiva. The initial stages usually consist of a few blood vessels growing in a triangular fashion [44]. This proceeds to definite recurrence where the blood vessels are accompanied by a variable amount of subconjunctival tissue. Sometimes a few blood vessels reach 1-2 mm onto the cornea (Gibson's vascularized scar).

"Any fibrovascular growth extending across the limbus onto the cornea at the site of surgical excision is defined as a recurrence". This may be more distressing to the patient than the primary one. The onset of recurrence was recorded as the date on which the recurrence was noted irrespective of the duration between that visit and the previous visit[36]. Majority of recurrences can be detected within the first 1 month period and almost all invariably by 6 months.

The risk factors for recurrence include geographic location, age and morphology of the pterygium. It has been noted that males, age < 40 years and those with vascular pterygia have higher recurrence rates. Bare sclera technique and primary closure have high recurrence rates and therefore should be avoided.

PART - II

AIM

To compare the efficacy and safety of amniotic membrane graft as a viable alternative to conjunctival autograft in pterygium surgery.

MATERIALS AND METHODS

70 Patients(70 eyes) presenting to the outpatient department of Stanley Medical Hospital, Chennai with Pterygium were included in this study.

Patients were randomly selected and divided into two groups of 35 each. One group underwent pterygium excision and replacement with conjuctival autograft using fibrin thrombin glue and the other group pterygium excision and replacement with amniotic membrane graft using fibrin thrombin glue.

Amniotic membrane was obtained from RSRM hospital and then processed and stored as per the guidelines.

The importance of surgical excision and the surgical procedure was explained to the patient following which informed consent was obtained.

Routine Pre operative evaluation done as per standards

1. Relevant ocular history.
2. Uncorrected and best corrected visual acuity
3. Slit lamp examination of type and extent of pterygium

4. Pre operative refraction
5. Fundus examination

SURGICAL STEPS

- Peribulbar anaesthesia was given
- Head of pterygium was separated from the corneal surface. After excision of the head and most of the body of pterygium, sub-conjunctival tissue was dissected and excised.
- Size of defect was measured and appropriate size conjunctival autograft was taken from superotemporal quadrant of the same eye and graft fixation was achieved using fibrin glue.
- For the amniotic membrane group, appropriate size amniotic membrane graft was used and placed with the basement side facing upwards. Graft fixation was achieved using fibrin glue.

Post operative

Conjunctival autogrft

Amniotic membrane graft

- Post operatively patient was prescribed antibiotic –steroid eye drops 6 times a day which was gradually tapered over 4 weeks.

POST OPERATIVE ASSESSMENT

Post operatively, patients were assessed on day 1, 1week, 1 month and 6months. Assessment included ocular symptoms, slit lamp examination of graft position and complications, refraction and recurrence.

INCLUSION CRITERIA

1. Patients above 20 years of age

2. Primary pterygium invading >2mm of cornea.

EXCLUSION CRITERIA

1. Eyelid disorders

2. Recurrent Pterygium

3. Pseudo pterygium

4. Anterior segment disorders

5. Prior history of ocular surgeries

OBSERVATIONS

Table – 1 Patient distribution

Number of Patients	Count	%
Conjuntival Autograft Group	35	50.00
Amniotic Membrane Graft Group	35	50.00

Figure – 1aBar diagram showing patient distribution

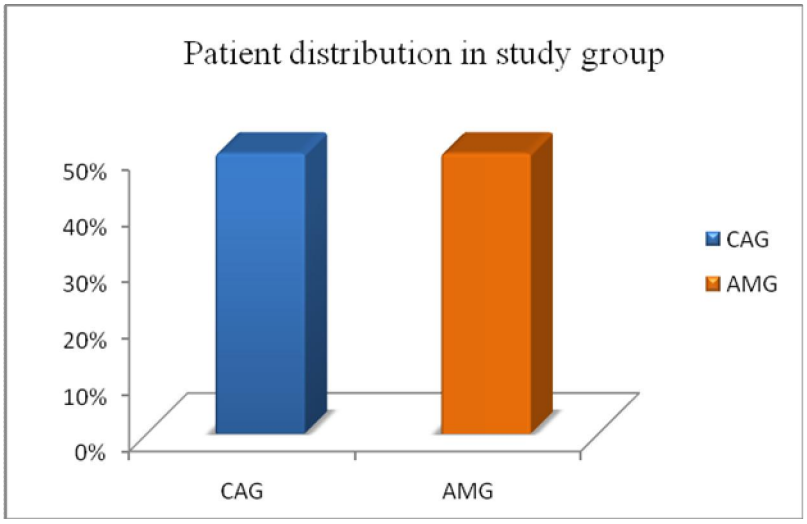
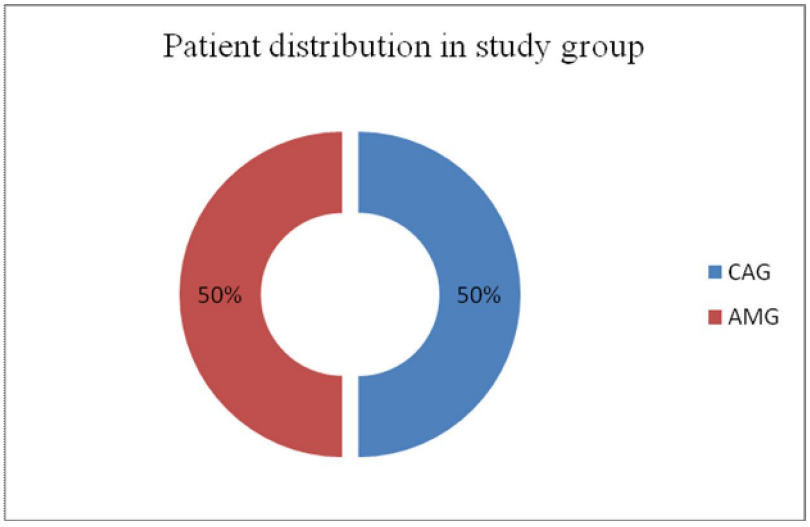


Figure – 1b Pie diagram showing patient distribution

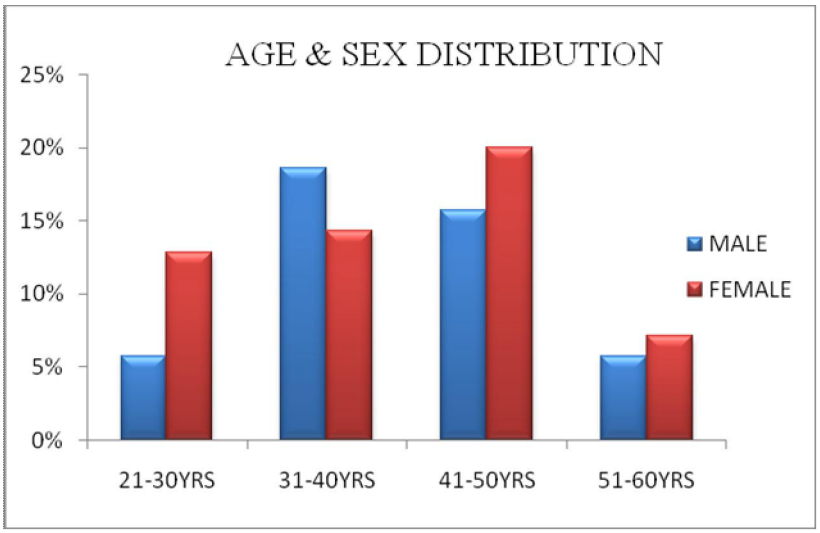


The chart shows that 50% of the patients were in the Conjunctival Autograft group (n = 35) and the remaining 50% belonged to the Amniotic membrane group.

Table – 2 Age and sex distribution

Age (Years)	Male	%	Female	%	Total	%
21-30	4	5.71	9	12.86	13	18.57
31-40	13	18.57	10	14.29	23	32.86
41-50	11	15.71	14	20.00	25	35.71
51-60	4	5.71	5	7.14	9	12.86
Total	32	45.71	38	54.29	70	100.00

Figure – 2a Bar diagram showing age and sex distribution



From the above data it is seen that the incidence of pterygium is highest in the third (32.86%) and fourth decades (35.71%) of life in both males and females.

Figure – 2b Pie chart showing age distribution in male patients

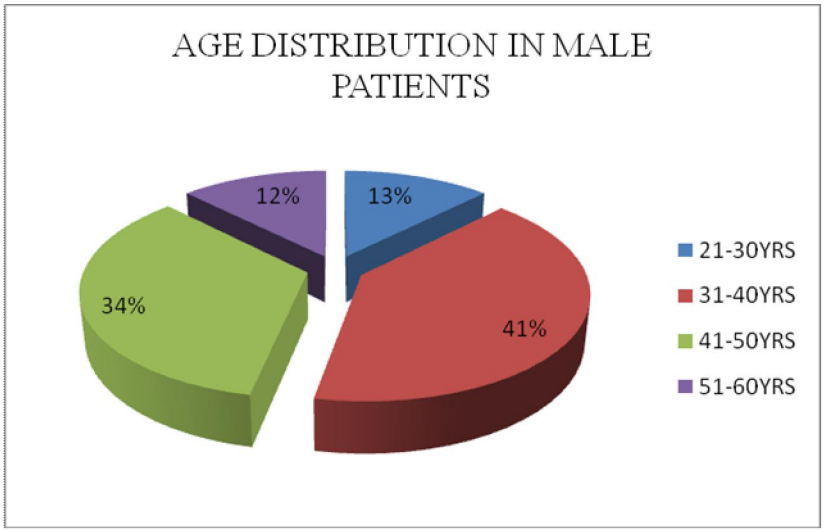
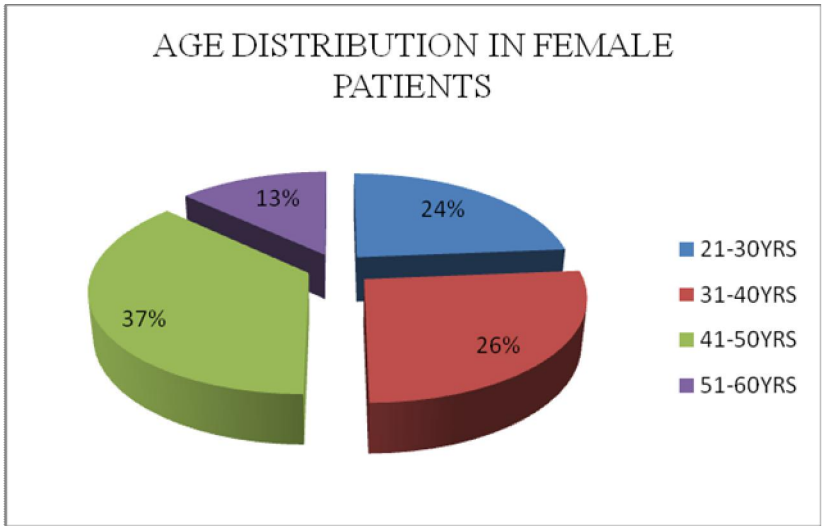


Figure – 2c Pie chart showing age distribution in female patients



The maximum occurrence of pterygium among the male patients was in the 31-40 yrs age group. Among the females the occurrence of pterygium was maximum in the 41-50 age group.

Table – 3 Gender distribution

	Male	%	Female	%
Conjuntival Autograft Group	13	18.57	22	31.43
Amniotic Membrane Graft Group	19	27.14	16	22.86
Total	32	45.71	38	54.29

The table shows that 45.71% of the affected patients were males and 54.29% of the affected patients were females in the entire study group.

Figure – 3a Bar diagram showing gender distribution in each group

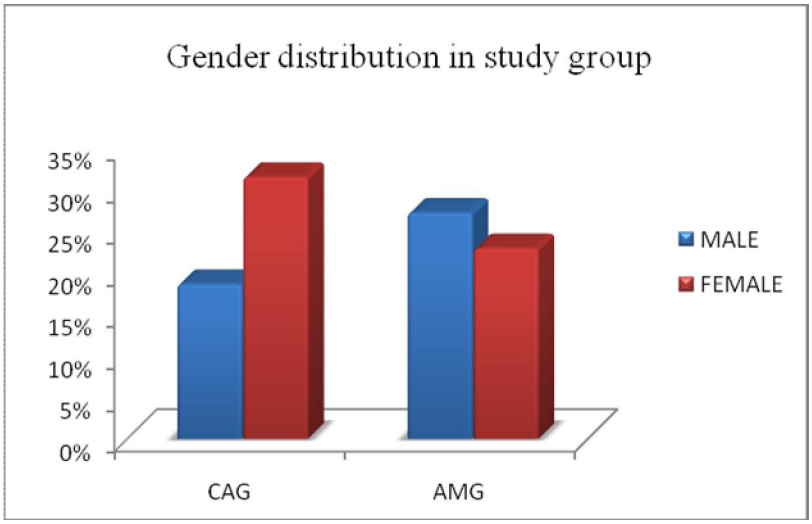


Figure – 3b Pie chart showing gender distribution in the conjunctival autograft group

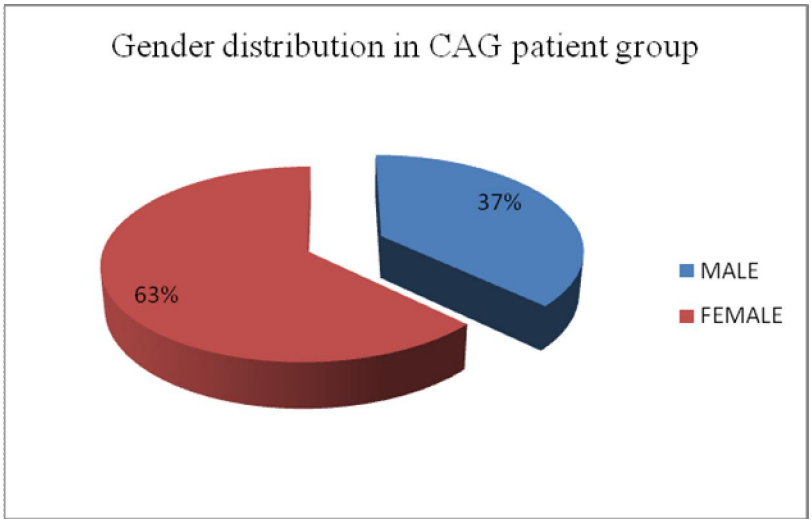
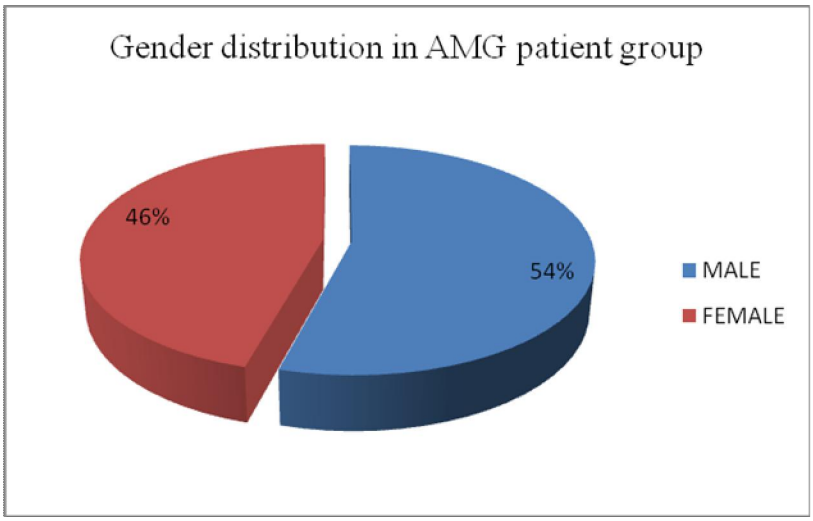


Figure – 3c Pie chart showing gender distribution in the amniotic membrane group



In the ConjunctivalAutograft group 37% were males and 63% were females. In the Amniotic membrane group 54% were male and 46% were female.

Table – 4 Occurrence of pterygium with respect to occupation

Occupation	No. of Patients	%
Farmer	12	17.14
Carpenter	3	4.29
Coolie	8	11.43
Watchman	6	8.57
Cook / Maid	10	14.29
Housewife	16	22.86
Vendor	15	21.43

Figure – 4a Bar diagram showing occurrence of pterygium with respect to occupation

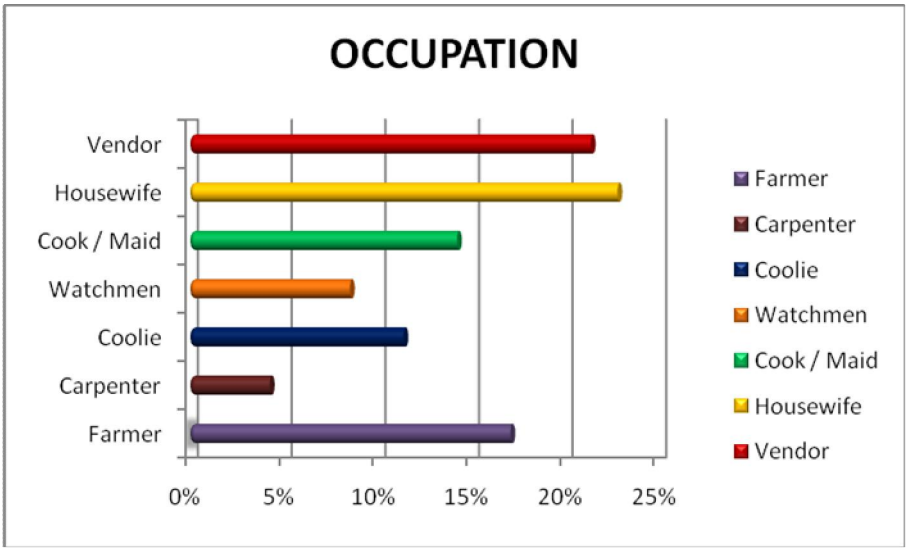
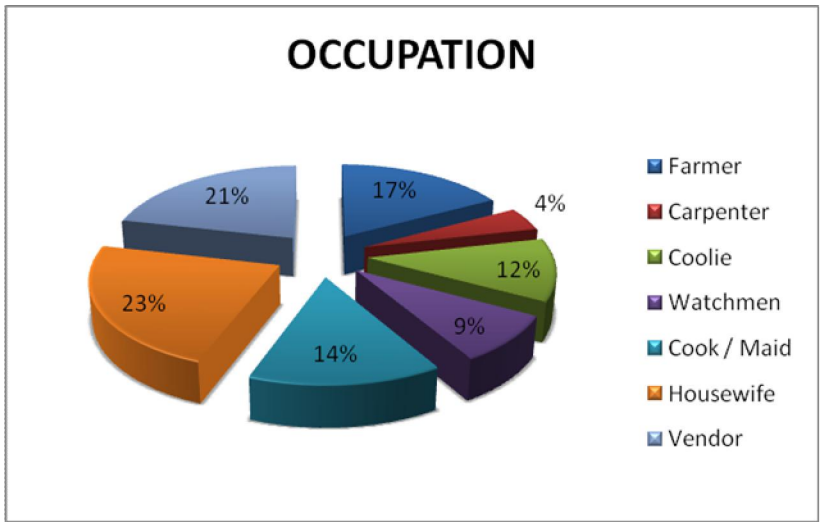


Figure – 4b Pie chart showing occurrence of pterygium with respect to occupation



In the entire study, pterygium was most prevalent in the outdoor occupation group with 62.85% of the affected patients belonging to this group. Among those working outdoors the most affected were farmers and vendors. The remaining 37.14% of the patients worked indoors.

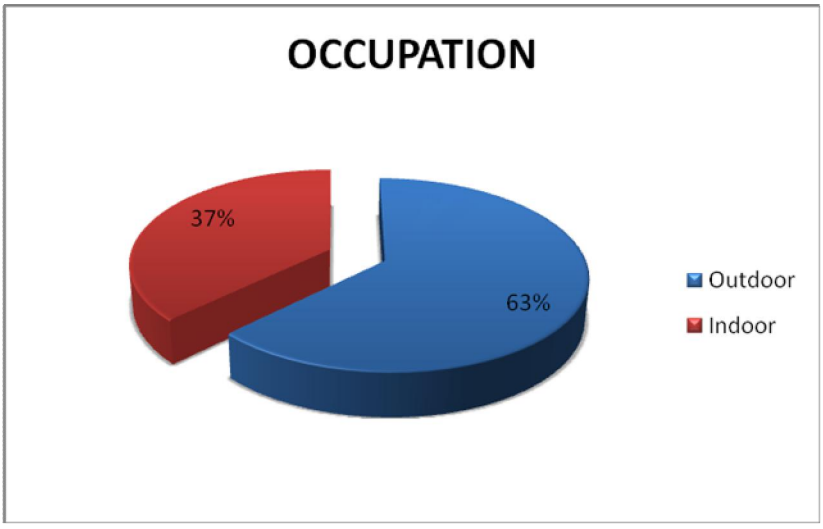


Table – 5 Laterality of pterygium

Latrality		No. of Patients	%
Unilateral	RE	25	35.71
	LE	22	31.43
Bilateral	BE	23	32.86

From the above table it is seen that among the study group, 47 patients had unilateral pterygium, while 23 patients had bilateral pterygium.

Figure – 5a Bar graph showing laterality of pterygium

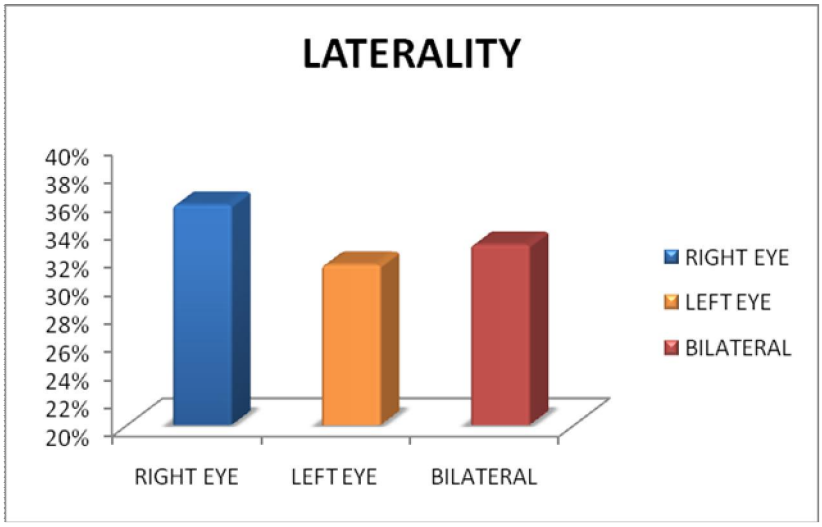
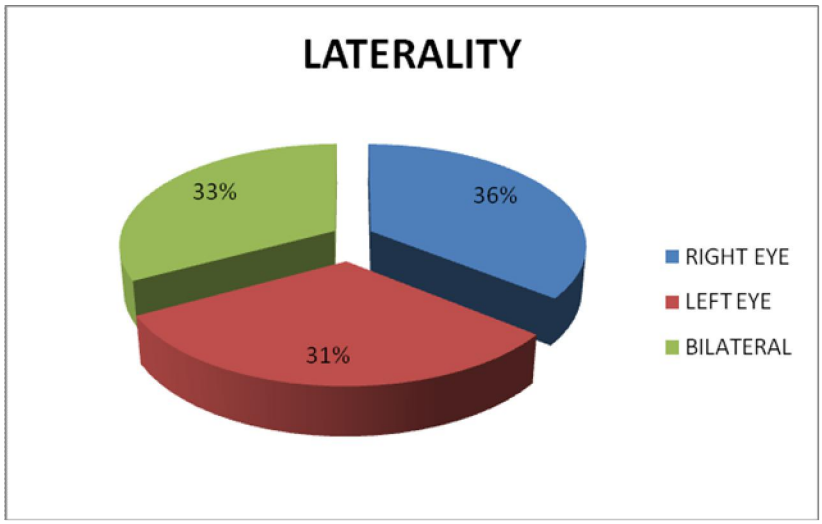


Figure – 5b Pie chart showing laterality of pterygium

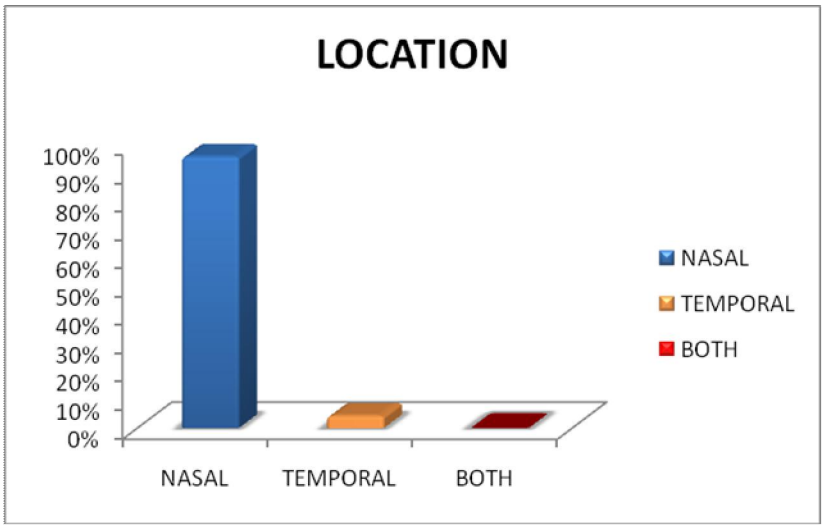


From the above chart, pterygium affected 36%, 31% and 33% of the cases in the right eye, left eye and both eyes respectively.

Table – 6 Location of pterygium

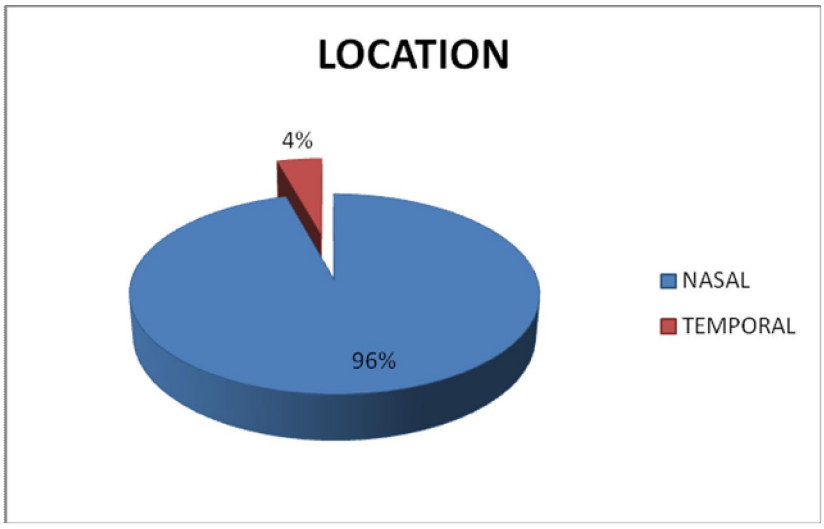
Location	No. of Patients	%
Nasal	67	95.71
Temporal	3	4.29
Both	0	0

Figure – 6a Bar diagram showing location of pterygium



Out of the 70 patients included in the study, 67 patients (95.71%) had nasal pterygium, and 3 patients(4.29%) had temporal pterygium. Incidence of double headed pterygium was 0%

Figure – 6a Pie chart showing location of pterygium



Incidence of nasal pterygium was much higher than that of temporal pterygium.

Graft edema

Following conjunctival autograft

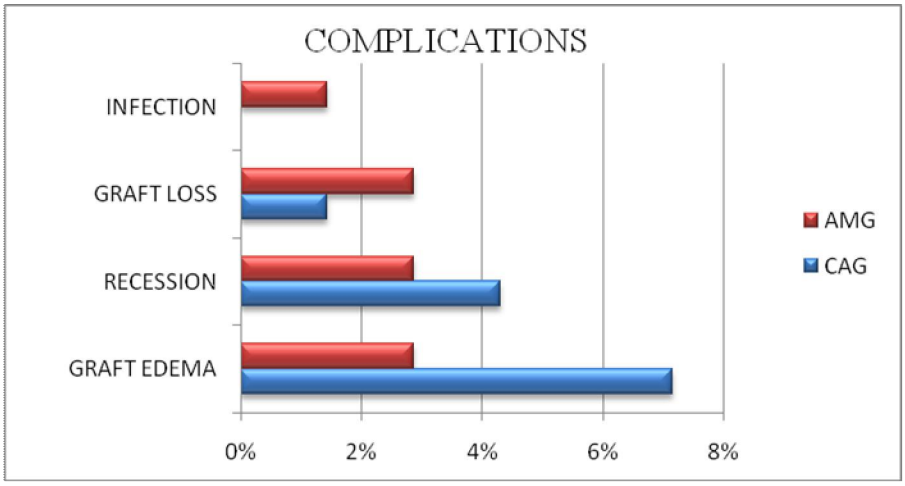
Post operative infection

COMPLICATIONS- EARLY POST OPERATIVE

Table – 7 Complications observed in the two study groups

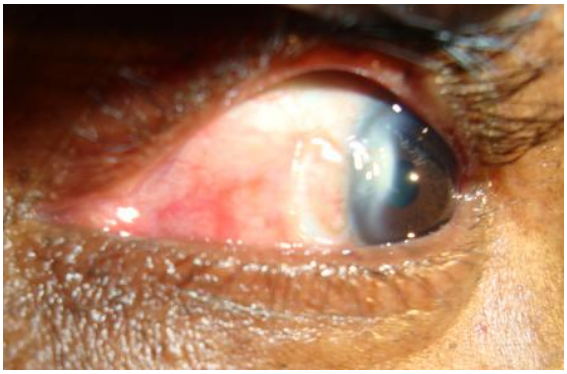
Complications	CAG		AMG	
	Count	%	Count	%
Graft Edema	5	7.14	2	2.86
Graft Edge Recession	3	4.29	2	2.86
Graft Loss	1	1.43	2	2.86
Infection	0	0.00	1	1.43

Figure – 7a Bar diagram showing complications observed in the conjunctival autograft and amniotic membrane group

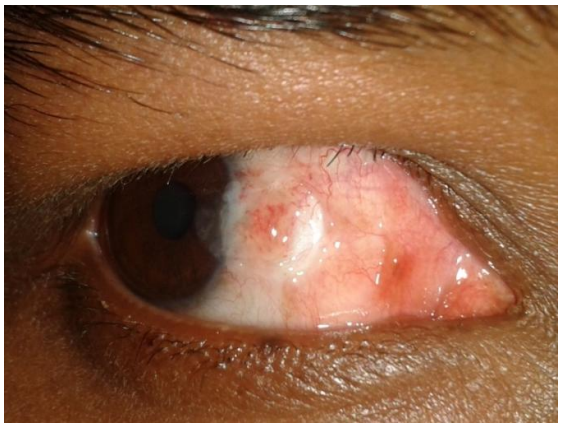


The above data shows that, Graft edema was observed in 5 patients in the conjunctival autograft group and 2 patients in the amniotic membrane group, in the immediate post operative period. P value - 0.231(no significant difference)

Graft edge recession



Conjunctival autograft



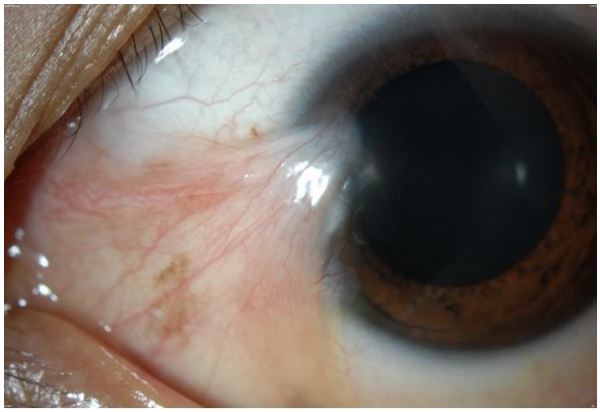
Amniotic membrane graft

Graft edge recession was noticed in 3patients and 2 patients in the conjunctival autograft and amniotic membrane group respectively. P value – 0.643(no significant difference)

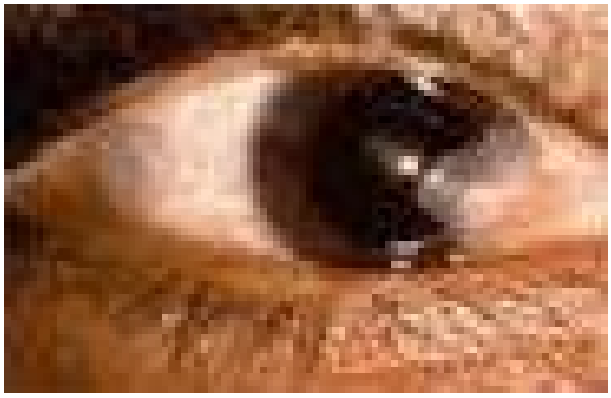
Total loss of graft tissue occurred in 1 patient in the conjunctival autograft group and 2 patients in the amniotic membrane group. P value - 0.555(no significant difference)

Post operative infection occurred only in 1 patient who belonged to the amniotic membrane group. P value - 0.314(no significant difference)

Recurrence



Conjunctival autograft



Amniotic membrane graft

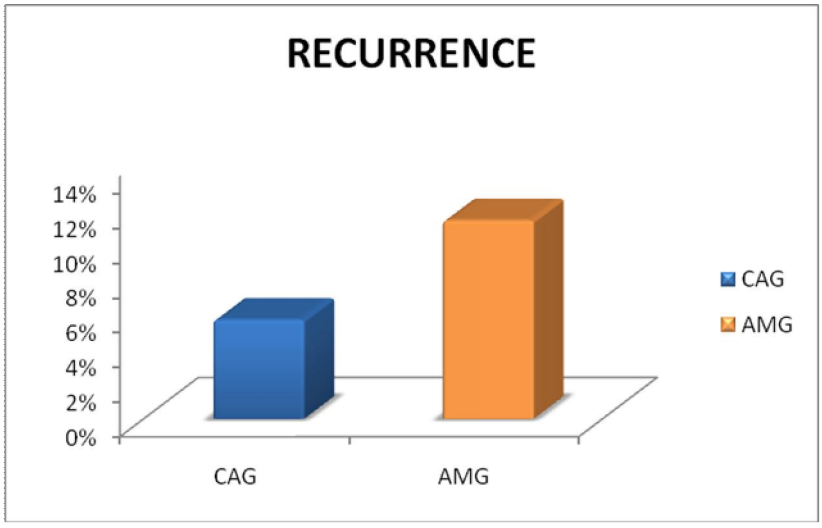
COMPLICATIONS- LATE POST OPERATIVE

Table – 8 Recurrence in the two study groups

Procedure	No. of Patients	No. of Recurences	%
Conjuntival Autograft Group	35	2	5.7143
Amniotic Membrane Graft Group	35	4	11.43

Recurrence of pterygium was seen among 2 patients in the conjunctival autograft group and among 4 patients in the amniotic membrane group.χ^2 =0.729 and P value = 0.393(> 0.05). There was no significant difference in the recurrence rates of the two study groups.

Figure – 8a Bar diagram showing recurrence of pterygium in conjunctival autograft and amniotic membrane group



Recurrence rate in the conjunctival autograft group and amniotic membrane group was 5.71% and 11.43% respectively

DISCUSSION

In India, pterygium is a common problem encountered by ophthalmologists in their day to day practice. Though surgery is the treatment of choice, it is associated with a high recurrence rate. Several surgical techniques evolved over the years, indicating the difficulty in finding one ‘ideal’ procedure.

Autologous conjunctival grafting is a simple and effective procedure and it does not involve loss of tissue. The low recurrence rate is attributed to the fact that normal conjunctiva acts as a barrier, preventing proliferation and advancement of the abnormal tissue towards the limbus. Inspite of the reduction in recurrences,one of the major concerns has been that it may affect the outcome of glaucoma-filtration surgery if required in the donor at a later date.In cases with double headed pterygium or extensive pterygium, this procedure may not be possible due to the dearth of healthy tissue for grafting.

Hence the need for a viable alternative arose and amniotic membrane was found to be a good option in this regard.

Amniotic membrane reduces recurrences by promoting conjunctival epithelialisation, inhibiting inflammation and suppressing sub conjunctival fibrosis. Hence, the relevance of a study comparing these two techniques.

Pterygium is more common in adults in the middle age group. In the present study, most of the patients were found to be in the age group of 41-50 years (35.71%). The next highest affected group was the 31-40 years age group (32.86%). The high incidence seen among these age groups may be attributed to occupational exposure.

Pterygium is more common in men than women probably due to greater exposure to dust and environmental factors. But if the individuals are involved in the same kind of occupation, this sex difference disappears as reported by J.H. Hillgers[56]. In the present study, out of 70 patients, 32 (45.71%) were males and 38 (54.29%) were females.

Occupation plays a major role in the aetiopathogenesis of pterygium. In the present study, pterygium was more common in persons engaged in outdoor occupations eg. farmers, coolies, vendors and they account for upto 44 out of the total 70 cases (62.85%). This is in accordance with the findings of MacReynolds[66], who stated that pterygium is more

common among farmers than those people employed in sedentary occupations. Similar studies have been published by Hillgers[56], Anderson and Kerknezov[67].

In the present study, 47 (67.14%) patients had unilateral pterygium of which right eye was affected in 25(35.71%) and left eye in 22(31.43%) of the patients. Bilateral pterygium was seen in 23(32.86%) of the patients.

In the present study 67(95.71%) of the patients had pterygium on the nasal aspect and 3(4.29%) on the temporal aspect. The nasal aspect of pterygium has been attributed to the fact that tears carrying dust particles flow from the temporal to the nasal aspect and accumulate thus causing greater irritation. This is in accordance with studies done by Shaw et al.

In the present study, graft edema was observed in 5(7.14%) and 2(2.86%) patients in the conjunctival autograft group and amniotic membrane group respectively. This subsided within one week with topical antibiotics and topical steroids.

Graft edge recession was noticed in 3(4.29%) patients in the conjunctival autogarft group and 2(2.86%) patients in the amniotic membrane group.

Loss of graft tissue occurred in 1 patient in the conjunctival autograft group and 2 patients in the amniotic membrane group.

Post operative infection did not occur in the conjunctival autograft group, whereas in the amniotic membrane group 1 patient developed post operative infection involving the cornea. Fungal pathogens were isolated. She received topical, systemic anti fungal medications and was referred to a higher centre where she underwent therapeutic keratoplasty.

In the present study, recurrence rate in the conjunctival autograft group was 5.71% and in the amniotic membrane group was 11.43%. These rates were similar to those obtained in other studies done by Kenyon[12], Solomon et al[68] and Prabhasawat et al[43]. In a study by Memarzadeh F, Fahd AK, Shamie N and Chuck RS, amniotic membrane transplantation was done in 23 eyes and conjunctival autografting was done in 40 eyes[69]. The pterygium recurrence rates after AM graft and conjunctival autograft were 35 and 25%, respectively.

CONCLUSION

- Pterygium is more commonly seen in the third (32.86%) and fourth (35.71%) decades of life in both males and females.
- Pterygium was more common among females (54.29%).
- Incidence of pterygium was found to be more in those engaged in outdoor occupations ie.62.85% of the total.
- Unilateral pterygium(67.14%) was more common than bilateral pterygium(32.86%).
- Nasal pterygium had almost 96% occurrence.
- Graft edema was seen more in the conjunctival autograft group (7.14%) than the amniotic membrane group (2.86%).
- Graft edge recession was seen more among the conjunctival autograft group (4.29 %).
- Loss of graft tissue was observed to be greater in the amniotic membrane group (2.86%).
- One patient developed post operative infection in the amniotic membrane group.

- Recurrence rate in the conjunctival autograft group was 5.71%.
- Recurrence rate in the amniotic membrane group was 11.43%.
- Recurrence rates between the two groups did not show any statistical significance ($p = 0.393$).

Autologous conjunctival grafting is a safe and simple procedure which is also associated with a low recurrence rate. It also has a lower incidence of complications. The disadvantage with conjunctival autografts include the need to use an operating microscope and the need for considerable surgical skill. This technique is of limited use in cases with double headed pterygium and in those cases with cicatrical disorders involving large areas of the conjunctiva. There is an associated risk that this procedure may affect the outcome of glaucoma filtration surgery if required in the future.

Amniotic membrane usage has shown encouraging results especially in clinical situations requiring extensive tissue repair, recurrent cases with scarring and in patients who need filtration surgery. It is also free of rejection. The major drawback however, is that its procurement under aseptic and sterile conditions is time consuming,

tedious and requires experience, expertise and financial resources. Storage and preparation of media requires trained personnel and good laboratory facilities.

Thus from the results of our study, I conclude that both amniotic membrane graft and conjunctival autograft methods are equally effective treatment options for pterygium surgery, with comparable recurrence rates and cosmetic results and either may be selected based on the patient characteristics and the facilities available to the ophthalmologist.

ANNEXURES

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PROFORMA CASE SHEET

Name Age IP No

Sex M ☐ F ☐

RE ☐ LE ☐

OCCUPATION

Total No of hours spent outdoors _____

Persons using Protection Yes ☐ No ☐

(Sun Glasses/Cap/Umbrella)

Presenting Complaints with Duration

- Ocular Irritation
- Recurrent Inflammation
- Visual impairment
- Cosmetic disfigurement

PAST HISOTRY

FAMILY HISTORY - Pterygium in family members

INVESTIGATIONS

BLOOD SUGAR

Hb

BT

CT

OCULAR EXAMINATION

		RE	LE
VISUAL ACUITY			
VA WITH PH			
EYELID			
EOM			
CONJUNCTIVA			
• Location of Pterygium	Nasal	☐	☐
	Temporal	☐	☐
• Type of pterygium	Progressive	☐	☐
	Atrophic	☐	☐
• Grading of pterygium	T1	☐	☐
	T2	☐	☐
	T3	☐	☐
• Size in Millimeters			

CORNEA

IRIS

PUPIL

LENS

TENSION

FUNDUS

PRE– OPERATIVE	RE	LE
REFRACTION		

INTRA OPERATIVE NOTES

(I) Anaesthesia - Peribulbar

(II) Surgery - Pterygium Excision

(III) Graft - Conjunctivalautograft / amniotic membrane graft

POST OPERATIVE ASSESSMENT

- Symptoms
- Slit Lamp Examination of graft
- Refraction

POST– OPERATIVE	RE	LE
REFRACTION		

சுய ஒப்புதல் படிவம்

ஆராய்ச்சி நிலையம் - அரசு ஸ்டான்லி மருத்துவமனை

சென்னை - 600 001.

பங்கு பெறுபவரின் பெயர் -

பங்கு பெறுபவரின் எண் -

பங்கு பெறுபவர் இதனை (√)குறிக்கவும்.

மேலே குறிப்பிடப்பட்டுள்ள வெண்படலமுனைத்திசு வளர்ச்சி அறுவை சிகிச்சை ஆய்வு விவரங்கள் எனக்கு விளக்கப்பட்டது. என்னுடைய சந்தேகங்களை கேட்கவும், அதற்கான தகுந்த விளக்கங்களை பெறவும் வாய்ப்பளிக்கப்பட்டது. ☐

நான் இவ்வாய்வில் தன்னிச்சையாகதான் பங்கேற்கிறேன். அந்த கட்டத்திலும் எந்த சட்ட சிக்கலுக்கும் உட்படாமல் நான் இவ்வாய்வில் இருந்து விலகிக் கொள்ளலாம் என்றும் அறிந்து கொண்டேன். ☐

இந்த அறுவை சிகிச்சை சம்பந்தமாகவோ, இதை சார்ந்து மேலும் ஆய்வு மேற்கொள்ளும் போதும் இந்த ஆய்வில் பங்குபெறும், மருத்துவர் என்றுடைய மருத்துவ அறிக்கைகளை பார்ப்பதற்கு என் அனுமதி தேவையில்லை என அறிந்து கொள்கிறேன். நான் ஆய்வில் இருந்து விலகிக் கொண்டாலும் இது பொருந்தும் என அறிகிறேன். ☐

இந்த ஆய்வின் மூலம் கிடைக்கும் தகவல்களையும், பரிசோதனை முடிவுகளையும் மற்றும் அறுவை சிகிச்சை தொடர்பான தகவல்களையும் மருத்துவர் மேற்கொள்ளும் ஆய்வின் பயன்படுத்திக் கொள்ளவும் அதை பிரசுரிக்கவும் என் முழு மனதுடன் சம்மதிக்கிறேன். ☐

இந்த வெண்படல முனைத்திசு வளர்ச்சி அறுவைசிகிச்சை ஆய்வில் பங்கு கொள்ள ஒப்புக் கொள்கிறேன் எனக்கு கொடுக்கப்பட்ட அறிவுரைகளின்படி நடந்து கொள்வதுடன் இந்த ஆய்வை மேற்கொள்ளும் மருத்துவ அணிக்கு உண்மையுடன் இருப்பேன் என்றும் உறுதியளிக்கிறேன். என் உடல் பாதிக்கப்பட்டாலோ அல்லது எதிர்பாராத வழக்கத்திற்கு மாறான நோய்க்குறி தென்பட்டாலோ உடனே அமை மருத்துவ அணிக்கு தெரிவிப்பேன் என உறுதி அளிக்கிறேன். ☐

பங்கேற்பவரின் கையொப்பம்.

ஆய்வாளரின் கையொப்பம்.

ABBREVIATIONS

CAG	-	Conjunctival autograft group
AMG	-	Amniotic membrane group
M	-	Male
F	-	Female
N	-	Nasal
T	-	Temporal
RE	-	Right Eye
LE	-	Left Eye
BE	-	Both Eyes
UNI	-	Unilateral
BI	-	Bilateral

MASTER CHART

Sl.No.	Name	Age	Sex	Hospital No.	Occupation	Uni/Bi	Nasal/Temporal	Procedure	Graft Edema	Graft Recession	Graft Loss	Infection	Recurrence	Complication
1	Anjali	51	F	20619	5	LE	N	CAG	-	-	-	-	-	Nil
2	Sridhar	50	M	27111	4	BE	N	CAG	+	-	-	-	-	Graft Edema
3	Murugan	33	M	27189	1	LE	N	CAG	-	-	-	-	-	Nil
4	Chengamal	60	F	25186	6	BE	N	CAG	-	+	-	-	-	Graft Recession
5	Meena	39	F	26649	7	RE	N	CAG	-	-	-	-	-	Nil
6	Kamatchi	50	F	26544	5	RE	N	CAG	-	-	-	-	-	Nil
7	Munusamy	55	M	20774	1	LE	T	CAG	+	-	-	-	-	Graft Edema
8	Lakshmi	50	F	20480	4	LE	N	CAG	-	-	-	-	-	Nil
9	Gyanamani	28	F	20482	5	RE	N	CAG	-	-	-	-	+	Recurrence
10	Siva	35	M	22588	3	BE	N	CAG	-	-	-	-	-	Nil
11	Annalakshmi	40	F	22613	6	LE	N	CAG	-	-	-	-	-	Nil
12	Vinodh	35	M	22614	3	RE	N	CAG	-	+	-	-	-	Graft Recession
13	Suseela	42	F	22917	6	LE	N	CAG	-	-	-	-	-	Nil
14	Meenatchi	24	F	22601	7	BE	N	CAG	+	-	-	-	-	Graft Edema
15	Devi	28	F	22596	5	RE	N	CAG	-	-	-	-	-	Nil
16	Pandiyan	52	M	22620	4	LE	N	CAG	-	-	-	-	-	Nil
17	Ramzan	30	F	24136	3	BE	N	CAG	-	-	-	-	-	Nil
18	Ameed	35	M	24140	7	BE	N	CAG	-	-	-	-	-	Nil
19	Anjali	48	F	24172	4	RE	T	CAG	-	-	+	-	-	Graft Loss
20	Banumathy	48	F	24135	6	BE	N	CAG	-	-	-	-	-	Nil
21	Navarthinam	42	M	24175	2	LE	N	CAG	-	-	-	-	+	Recurrence
22	Uma	30	F	25064	7	LE	N	CAG	-	-	-	-	-	Nil
23	Venilla	39	F	25049	6	RE	N	CAG	-	+	-	-	-	Graft Recession
24	Gomathi	32	F	25063	6	BE	N	CAG	-	-	-	-	-	Nil
25	Desamma	45	F	25055	5	BE	N	CAG	-	-	-	-	-	Nil
26	Manikala	35	F	25321	7	BE	N	CAG	-	-	-	-	-	Nil
27	Malar	35	F	25097	5	RE	N	CAG	-	-	-	-	-	Nil
28	Selvi	31	F	26349	6	LE	N	CAG	-	-	-	-	-	Nil
29	Rameza	28	F	26402	7	LE	N	CAG	-	-	-	-	-	Nil
30	Selvam	38	M	25612	1	LE	N	CAG	+	-	-	-	-	Graft Edema
31	Manovathy	30	F	25612	3	RE	N	CAG	+	-	-	-	-	Graft Edema
32	Murugan	35	M	25613	1	BE	N	CAG	-	-	-	-	-	Nil
33	Haribabu	41	M	27060	3	LE	N	CAG	-	-	-	-	-	Nil
34	Govindaraj	48	M	25545	1	RE	N	CAG	-	-	-	-	-	Nil
35	Kuppan	34	M	28471	1	BE	N	CAG	-	-	-	-	-	Nil

36	Muthulakshmi	43	F	27030	6	RE	N	AMG	-	-	-	-	-	Nil
37	Balaguru	49	M	29050	7	RE	N	AMG	-	-	-	-	+	Recurrene
38	Jeyalakshmi	44	F	29013	7	LE	N	AMG	-	-	-	-	-	Nil
39	Thilagam	56	F	30260	6	RE	N	AMG	-	+	-	-	-	Graft Recession
40	Bujjiammal	42	F	30231	6	BE	N	AMG	-	-	-	-	-	Nil
41	Nagarajan	47	M	30262	7	LE	N	AMG	-	-	-	-	-	Nil
42	Pushpa	43	F	30256	6	BE	N	AMG	-	-	+	+	-	Graft Loss, Infection
43	Dhanasekar	27	M	30609	3	RE	N	AMG	-	-	-	-	-	Nil
44	Thangadurai	40	M	30276	4	BE	N	AMG	+	-	-	-	-	Graft Edema
45	Sandavel	31	M	16772	2	RE	N	AMG	-	-	-	-	-	Nil
46	Vadivel	32	M	31246	1	BE	N	AMG	-	-	+	-	-	Graft Loss
47	Amudha	38	F	32202	7	BE	N	AMG	-	-	-	-	-	Nil
48	Hemavathy	40	F	16712	6	LE	N	AMG	-	-	-	-	-	Nil
49	Prakash	43	M	31280	7	RE	N	AMG	-	-	-	-	-	Nil
50	Saravanan	30	M	28174	7	RE	N	AMG	-	-	-	-	-	Nil
51	Desam	42	F	28189	5	LE	N	AMG	-	+	-	-	-	Graft Recession
52	Arul	29	M	27858	3	RE	N	AMG	-	-	-	-	-	Nil
53	Madhi	36	M	27863	3	LE	N	AMG	-	-	-	-	+	Recurrence
54	Kalavathi	44	F	28418	5	BE	N	AMG	-	-	-	-	-	Nil
55	Shakila Bee	50	F	28674	6	RE	N	AMG	-	-	-	-	-	Nil
56	tamilarasan	41	M	27625	1	BE	N	AMG	-	-	-	-	-	Nil
57	Srinivasan	32	M	27345	1	RE	N	AMG	-	-	-	-	-	Nil
58	Damayanthi	38	F	28194	6	LE	N	AMG	-	-	-	-	-	Nil
59	Ramachandran	35	M	28173	1	LE	N	AMG	-	-	-	-	+	Recurrence
60	Luthr Mary	58	F	28940	6	RE	T	AMG	-	-	-	-	-	Nil
61	Arivarasan	46	M	28659	7	BE	N	AMG	-	-	-	-	-	Nil
62	Shanthi	28	F	27850	6	RE	N	AMG	-	-	-	-	-	Nil
63	Ramasamy	41	M	27863	2	LE	N	AMG	-	-	-	-	+	Recurrence
64	Paravathy	46	F	28401	7	BE	N	AMG	-	-	-	-	-	Nil
65	Prakash	30	M	28418	1	RE	N	AMG	-	-	-	-	-	Nil
66	Goutham	41	M	28174	1	BE	N	AMG	-	-	-	-	-	Nil
67	Sudha	55	F	28189	5	RE	N	AMG	+	-	-	-	-	Graft Edema
68	Sudhakaran	60	M	25931	4	LE	N	AMG	-	-	-	-	-	Nil
69	Revathy	28	F	25493	5	RE	N	AMG	-	-	-	-	-	Nil
70	Ravi	54	M	26354	7	BE	N	AMG	-	-	-	-	-	Nil

Farmer-1
Carpender-2
Coolie -3
Watchman-4
Cook/Maid-5
House Wife-6
Vendor-7